The Journal of Laboratory and Clinical Medicine

VOL. 21 APRIL, 1936 NO. 7

CLINICAL AND EXPERIMENTAL

OBSERVATIONS ON THE pH OF CLOTTING AND CITRATED BLOOD*

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THE present observations have been made with the aid of a modified DuBois glass electrode[2] and electrode chamber[3] designed for determining the pH of blood with adequate prevention of CO_2 loss. Voltage was read with the DuBois potentiometer,[2] slightly modified. Preliminary tests on oxalated blood samples convinced us that the voltage readings remained reasonably constant over at least one-half hour. It was, therefore, believed possible to secure a series of readings on a blood sample introduced rapidly and allowed to clot while in the apparatus.

pH Determinations During Blood Clotting.—Blood was collected under paraffin oil in an all glass syringe. Rabbit heart blood was usually selected but a few determinations were also made on dog blood (femoral artery) and on the venous blood of decerebrate cats. The sample was transferred (without exposure to the air) to the glass electrode chamber, the whole procedure from the commencement of the blood taking to the first voltage reading occupying somewhat less than two minutes. Potentiometric readings were commenced immediately and repeated several times each minute over a period averaging fifteen or thirty minutes. The blood chamber was maintained at 38° C. by the flow of warmed water (*op. cit.*). Portions of each blood sample were allowed to clot in coagulation tubes and the times of commencing and complete clotting were noted as recorded under "clotting time" in the accompanying selected protocols. Since the results were as consistent as the delicacy of the instru-

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Received for publication, May 16, 1935.

mental manipulations would permit, the few cases cited in Table I sufficiently illustrate the data obtained. The absence of significant variation in pH during blood clotting is apparent from the figures quoted.

TABLE I

pH MEASUREMENTS DURING CLOTTING OF RABBIT'S HEART BLOOD

EXPER.	TIME (MINUTES) AFTER WITHDRAWAL OF BLOOD											CLOTTING TIME
	2′	4′	6′	8′	10′	12′	14′	16′	18′	20′	30′	
I	7.510	7.510	7.510	7.510	--	--	--	--	--	--	7.507	3½- 4½ min.
II	7.153	7.153	7.157	7.160	7.165	7.169	7.171	7.175	7.180	7.183	--	11½-12 min.
III	7.516	7.516	7.520	7.520	7.516	7.514	--	--	--	--	--	4 - 5 min.
IV	7.24	7.25	7.25	7.25	7.23	7.22	7.20	7.20	--	--	--	2 - 2½ min.

pH changes in (a) defibrinated and (b) citrated blood preserved in tonometers at room temperature. In these experiments the blood sample was divided between a series of mercury tonometers. In the first tonometer it was shaken until defibrinated. In the others it was mixed thoroughly with sodium citrate solution previously introduced above the mercury. After several trials with varying strengths and proportions of citrate, 0.5 c.c. of 10 per cent sodium citrate solution to 2.5 c.c. of blood was selected as a routine procedure. pH determinations were made with the glass electrode under the temperature conditions noted in the previous experiments. The tonometers were kept at room temperature (circa 24° C.) for twenty-four hours, small samples being removed at intervals for pH determinations. In every instance great care was taken throughout to avoid any gaseous exchange with the air, the technic being entirely reliable. On a few occasions, however, the test sample was subsequently aerated (in the electrode chamber) and pH was redetermined after equilibration with room air. Table II summarizes a few typical experiments.

TABLE II

pH MEASUREMENTS ON DEFIBRINATED AND CITRATED RABBIT'S HEART BLOOD*

EXPER.	DEFIBRINATED	CITRATED	DEFIBRINATED (AERATED)	CITRATED (AERATED)
I	(10 min.) 7.44	--	(15 min.) 8.13	--
	(3½ hr.) 7.35	(3 hr.) 7.20	(3¾ hr.) 7.94	(3½ hr.) 7.83
	(4¾ hr.) 7.32	(4½ hr.) 7.20	(5½ hr.) 7.80	(4½ hr.) 7.74
	(24 hr.) 7.08	(24 hr.) 6.95	--	--
II	(¾ hr.) 7.52	(¼ hr.) 7.34	--	--
	(1¾ hr.) 7.48	(1¼ hr.) 7.31	--	--
	(8¼ hr.) 7.27	(8 hr.) 7.13	--	--
	(21½ hr.) 7.13	(21 hr.) 7.08	--	--
III†	(2 min.) 7.510	--	--	--
	(½ hr.) 7.507	--	--	--
	--	(2 hr.) 7.33		(2½ hr.) 7.74

*Times (after withdrawal of blood) are indicated in parentheses.

†In this experiment, which is the same as No. I of Table I, the pH of the clotting blood (constant for ½ hr.) was used instead of defibrination.

Tests on Citrate Solutions.—A. Trisodium citrate solutions of strengths varying between 2.5 per cent and 10 per cent, some freshly prepared and some aged several months, were tested with the Leeds-Northrop H-electrode and found to have pH values ranging between 7.5 and 8.5. This slight alkalinity is in ac-

cordance with the feeble hydrolytic dissociation of the organic salt (v. infra). Citrate solutions are known to have but little buffer value (a fact not taken into account by Mellon, 1922) and some simple tests were set up to illustrate this point.

B. Two test tubes, each containing 10 c.c. of the usual phosphate buffer mixture at pH = 7.2 + 5 drops of 0.2 per cent phenol red indicator, were placed in Cole-Onslow comparator along with another similar tube containing a like amount of phosphate and indicator at pH, 7.4. A four-month-old 10 per cent solution of sodium citrate was run into one of the pH, 7.2 tubes and a corresponding amount of distilled water into each of the other tubes. After the addition of 6 or 7 c.c. of the citrate solution a color change became detectable (as compared with the control). Some 14 or 15 c.c. of citrate was required before the color matched that in the (watered) pH, 7.4 tube.

C. A specimen of rabbit venous blood (collected under oil) was subdivided between tonometers containing, respectively, (a) one-half, (b) twice, and (c) five times as much of (the same) 10 per cent sodium citrate as of blood. The corresponding pH values, with the glass electrode (no gas leakage), were (a) 6.91, (b) 7.01, and (c) 7.03. Although time intervals were not recorded in this experiment, which, therefore, takes no cognizance of the "acid shift" noted above, nevertheless, the determinations were all made within an hour or two and, in all probability, do indicate the alkalinizing effects of *large* amounts of citrate solution.

DISCUSSION

I. *pH During Blood Coagulation.*—Höber (1903) could find no pH change during blood clotting. He used the type of hydrogen electrode with which Michaelis (1914) recorded an acid-shift due to "the deposition of fibrin on the platinum." Ross (1921), using the dialysis technic of Dale and Evans (1920) which employed an indicator method said to be "of the same order of accuracy as the H-electrode," found no change (within ± 0.02 pH) in oxalated blood before and after recalcification. Kugelmass (1922), using a H-electrode, reported an "alkaline shift" of pH = 0.3 during the clotting of diluted oxalated plasma and prepared fibrinogen by a purified thrombin solution. He made no effort to control (*inter alia*) the gas equilibria. Hirsch (1924) used a delicate colorimetric method of determining pH by means of an indicator (cresol red) and the Keuffel-Esser color analyzer. Representative color readings were 51 per cent at pH = 7.45 and 60 per cent at pH = 7.36. The quoted pH values represent the average determinations on plasma and serum (respectively) in 10 samples of rabbit heart blood collected, centrifuged, and examined under a layer of paraffin oil to prevent gas loss. The acid change, averaging 0.09 pH, was not observed prior to clotting in 3 cases which failed to coagulate in half an hour, but it was detected before that time in several samples which clotted early. Havard and Kerridge (1929), using the glass electrode (Kerridge's pattern, 1926) to investigate acid changes in shed blood (see later) noted, incidentally, that clotting in the electrode pipette made no difference to the pH:time curves obtained. In some experiments at room temperature the pH remained constant during coagulation. Laug, 1934 (v. infra), with the Stadie

(1931) glass electrode, also recorded that clotting in the apparatus failed to modify these "acid-shifts." Eagle and Baumberger (1935) used the glass electrode of Baumberger to demonstrate the absence of pH change in the clotting of fibrinogen by thrombin.

Our experience with the DuBois glass electrode follows up the lead of these recent workers and helps to establish beyond a reasonable doubt that no significant change in pH (with instruments able to register accurately to within 0.005 or 0.01 of a pH unit) results from blood coagulation. Dismissing Kugelmass' anomalous and poorly controlled data, it would seem reasonable to explain Hirsch's results on the basis of the "acid-shift" which we shall review in the succeeding section.

II. *The "Acid-Shift" in Shed Blood.*—Lovatt Evans (1922) reviewed the earlier literature, dating from Zuntz (1868), to show that the CO_2 capacity of shed blood was highest immediately after withdrawal and usually suffered considerable reduction when the blood was allowed to stand outside the body. He gave good evidence that the chief cause of the phenomenon was the accumulation of lactic acid due to glycolysis. The "acid-shift" was irregular in incidence and magnitude. It did not occur in separated plasma but only in the presence of corpuscles, especially the leucocytes (Maclean and Weir, 1915). It was accelerated by previous lowering of the CO_2-tension (Mellanby and Thomas, 1920; Henderson and Haggard, 1920). Evans found that temperature played an important rôle. An initial chilling of the blood greatly retarded glycolysis and the acid change. Even without cooling it could be checked for at least half an hour by 0.4 per cent of oxalate (Macleod, 1913) plus 0.1 per cent of fluoride. A small correction (0.9 vols. per cent for each 0.1 per cent NaF) was necessitated by the reduction in CO_2 capacity by the added fluoride itself.

Havard and Kerridge (1929), without disturbing the gas equilibria, measured the pH of whole blood (at 38° C.) using Kerridge's (1926) modification of the bulb-type glass electrode. They, too, observed an acid-shift averaging pH = 0.05 which set in within two or six minutes (sometimes longer) after the blood had been drawn and held in the electrode. Clotting did not alter the pH:time curves. Blood corpuscles were necessary, the serum and plasma showing no pH variations, although centrifuged laked blood did. The acid change was markedly retarded at room temperature and completely stopped at 0° C. It was not affected by substances which inhibited (a) coagulation (oxalate, fluoride, heparin), and (rather anomalously) (b) glycolysis (NaF, 0.06 per cent). After a flattening out of the pH:time curves a later acid drift developed, leading them to emphasize the earlier alterations as a "first acid change." A few indefinite attempts at lactic acid estimations (and the foregoing considerations) caused them to avoid any correlation of the acid change with Evans' glycolytic reaction.

Laug recently reinvestigated the problem with the aid of Stadie's (1931) glass electrode and careful controls. He could not separate two phases of acid production but found that the change occurred from the commencement of observation (two and one-half minutes after withdrawal of blood) and amounted to an average (41 samples of whole blood at 38° C.) of 0.008, 0.007, 0.004,

0.004 (of a pH unit) in successive five-minute intervals. The data pointed to a continuous "acid-shift" running parallel with lactic acid production. The fact that the change was most marked in the first ten minutes suggested that glycolysis was especially vigorous at the outset. Fluorides were able to check the acid change if present in sufficient concentration but 1 or 2 per cent might be required instead of the 0.05 or 0.1 per cent concentrations usually employed. He again confirmed the importance of temperature, but gave good arguments against the possibility that the acid-shift was a mere "temperature artefact" as suggested by Platt and Dickinson (1933). Laug also adduces evidence which, on the whole, negates the possibility that electrical charges due to red blood cell sedimentation ("sedimentation artefact") play any significant rôle in the acidity phenomenon, although a definite drift, attributed to this cause, complicated some of his readings.

The DuBois pipette electrode in its present form is a modification of the MacInnes glass electrode. It has an active diameter of less than 0.5 mm. and a very low *resistance,* averaging below 5 megohms. For blood measurements the outside of the electrode tip is ground to fit snugly into the top of the capillary tube containing the blood. The blood chamber is kept at 38° C. by a warming jacket permitting a flow of water at the desired temperature, and temperature fluctuations of the blood sample, when transferred immediately, as in the present experiments, are minimal. The plugging of the blood tube by the pipette prevents the escape of CO_2, and, since the glass membrane is at the top of the blood column, its potential is unaffected by the drift or settling of the red blood cells. We feel, therefore, that the artefacts discussed by Laug may be ruled out in our experiments. The present data agree with those of Laug and of Havard and Kerridge in identifying a true "acid-shift" in shed blood. In the light of the literature cited, albeit the possibilities of oxidative phenomena and bacterial action have not received serious consideration, we may interpret the acid change in terms of a glycolytic phenomenon.

Our additional finding of a slight acidifying effect of small quantities of trisodium citrate bears out the recent work of Hastings, McLean, Eichelberger, Hall and DaCosta (1934) on the ionization of calcium citrate. Estimating calcium ion concentration by the new frog heart technic, they demonstrated clearly that calcium citrate ionizes in two stages. The primary dissociation is almost complete and follows the equation:

$$Ca_3Cit_2 = Ca(CaCit)_2 \rightleftharpoons Ca^{++} + 2(CaCit)^{-}.$$

The secondary dissociation, being that of a weak electrolyte, is incomplete, and according to the equation:

$$CaCit^{-} \rightleftharpoons Ca^{++} + Cit^{=}.$$

At 22° C., pH = 7.4, and total ionic strength of $\mu 0.155 - \mu 0.163$, the electrolytic dissociation constant, $pK_{CaCit^{-}}$ was computed to be 3.22 ± 0.025. The scanty data in the literature on calcium citrate conductivity were quoted by these authors in support of the idea that such complex citrate ions do exist.

Explicable also on the basis of the foregoing data is the observation of Greenberg and Greenberg (1932) that citrates caused calcium to migrate to the anode in electrodialysis of serum ultrafiltrates.

The alkalinizing effects of *large* quantities of citrate probably depend upon interionic equilibria such as are invoked to explain loss of calcium by precipitation during plasma determinations (Cameron and Moorhouse, 1925).

SUMMARY

1. pH determinations with the sensitive glass electrode demonstrate no change during blood coagulation.

2. The pH of blood samples preserved in mercury tonometers at room temperature, with no attention to bacteriologic sterility, glycolysis, or oxidative phenomena, shows a pronounced "acid-shift" during a twenty-four-hour observation period. There are indications that the change may commence within half an hour. Data in the literature point to lactic acid production by glycolysis as the major cause of the acid change.

3. Trisodium citrate in small quantities (used as an anticoagulant) consistently gave samples slightly more acid than untreated (clotted) blood preserved for corresponding periods. This is explained on the basis of a primary dissociation into $CaCit^-$ anions.

4. The "acid-shifts," due to both time and citration, persisted in samples subsequently alkalinized by aeration.

5. H-electrode determinations of the pH of various citrate solutions gave values ranging between 7.5 and 8.5. An old 10 per cent solution was sufficiently alkaline for proportions (citrate:diluent) in excess of 1:2 to give an "alkaline shift" to (a) phosphate buffer mixture and (b) rabbit venous blood.

Besides settling a controversial question concerning blood coagulation, these data have several practical applications, first, to the preservation of blood samples for pH determination, and second, in connection with the problems of human blood transfusion by the citrate method.

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AURICULAR FLUTTER WITH 1:1 RESPONSE*

A Case Report

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AURICULAR flutter, although more rarely encountered, is closely related to auricular fibrillation. Flutter is believed[1] to result from "circus movement," i.e., a wave of contraction circulating regularly and continuously through the auricle. Usually, if untreated, it continues unchanged for months or years.[6] Instances are known[1] in which the auricles beat without cessation at a regular rate of 280 per minute for a period of ten or more years, a 2:1 block causing a ventricular rate of half the above, the patient meanwhile showing little change. Rarely it occurs in short paroxysms.[1] In Table I, "established flutter" indicates failure of the arrhythmia to end spontaneously within fourteen days,[6] "paroxysmal flutter" denotes briefer attacks.[5] Symptoms of this persistent condition resemble those of auricular fibrillation. The prognosis in flutter is much the same as in fibrillation. The reaction of the heart depends on its reserve and on the nature of the burden imposed by the flutter.[1] It is important to remember that flutter is very amenable to proper treatment.

The auricular rate in flutter is usually 260 to 320 per minute.[1] The ventricles commonly respond to each alternate auricular beat: the ventricular rate being half the auricular rate or 130 to 160 per minute. A certain degree of block is usually associated. This varies. There may be complete auriculoventricular dissociation,[2, 3] 4:1, 3:1, 2:1 block, while infrequently each auricular impulse provokes a contraction of the ventricles. The ventricular rate becomes the same as that of the rapidly beating auricles.

A patient with 2:1 flutter, although conscious of palpitation, is often able to lead practically a normal life. He may, however, suddenly become a victim of these paralyzing attacks of 1:1 flutter. Such 1:1 response occurs whenever the refractory period of the A-V node becomes less than the length of one auricular cycle.[5] These paroxysms at first may occur only once a week. Gradually they become more frequent, requiring less and less to precipitate them. Finally they occur daily or oftener, even with the patient at rest in bed.[18] In 1:1 flutter, the junctional tissues have been known to transmit stimuli up to a rate of 320 per minute,[4] the fastest human ventricular rate ever recorded. It is a higher rate of frequency than is recorded in any other type of cardiac arrhythmia.[5]

In a critical and exhaustive survey of the literature, Bedell[5] reports 16 instances of authentic auricular flutter with 1:1 response. To these she adds two additional cases. This is preponderantly a series of middle-aged pa-

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Received for publication, May 20, 1935.

tients.[5] Sixteen patients were males and three were females, bearing out the statement of Lewis[7] that flutter is four times as common in males as in females. The frequency of cases with 1:1 attacks among all patients with auricular flutter is reported to be 4 per cent.[5, 6] The etiologic factors are the same as those responsible for flutter in general: rheumatism, arteriosclerosis, syphilis, hypertension, hyperthyroidism, or any other disease resulting from toxin, infection, or otherwise. A 2:1 block predominated in all of these patients with 1:1 paroxysms.

The attacks usually follow some form of exertion or excitement. Paroxysms have followed the administration of quinidine[5, 6] or a combination of morphine, atropine, and avertin.[5] The onset is sudden with an immediate jump from 2:1 to 1:1 response. Extreme weakness, palpitation and shortness of breath are the usual manifestations. These symptoms force the ambulatory patient to sit down or may cause the patient in bed to sit up in order to breathe. The palpitation is usually overshadowed by the other symptoms and may be overlooked by the patient in giving the history of an attack. Precordial pain may be present as in Cases 3, 11, and 16. It may radiate to the epigastrium or down the left arm. Tremor, sweating, vertigo, nausea, and great mental agitation are frequently felt. Usually syncope does not occur but consciousness may be lost, as in Cases 7, 12, and 15. Congestive failure is not common but may occur as in Case 7. The patient is usually unable to stir during the attack, Case 4 being a remarkable exception. Paroxysms are usually transitory. In the majority of instances their duration is from thirty minutes to two hours. An attack may last only a few minutes or a number of hours. The offset of an attack is usually gradual. The two grades of block intermingle. A gradual transition from one heart rate to the other is thereby provided. Most attacks end spontaneously. At times therapy has been effective as in Cases 5, 7, 15, and 19. Bedell[5] reports the only instance of 1:1 flutter, established by electrocardiograms, in which a complete autopsy has been performed. The attacks of syncope and the possibility of congestive failure during frequent paroxysms somewhat modify the general prognosis in flutter.[5] The most important remedy in flutter is digitalis or an allied drug.[1] Digitalis effectively prevents 1:1 paroxysms by increasing the A-V block.[5] "Quinidine employed in flutter enjoys no great success."[1] It has been held responsible for paroxysms of 1:1 response in flutter.[5, 6] Flutter was effectively controlled by quinidine in the case here reported.

CASE HISTORY

White, male, aged sixty years, was admitted to Cook County Hospital, May 17, 1933. He complained of attacks of palpitation following influenza one year previously. The attacks became more frequent and had confined him to bed for several months because of their daily recurrence. These paroxysms usually followed exertion: walking, straining at stool, or sitting upright in bed. The attacks were of sudden onset, the heart continuing to pound until he would lie down, after which there would be a couple of very "large thumps" and then the attack would subside altogether. The patient himself discovered that flexing the head and putting his hand against the carotid artery stopped the attacks. Vertigo was noticed during these attacks. There were no other complaints. He had been confined to bed for several weeks with rheumatism at the age of eleven years. He admitted having had gonorrhea at the age of thirty years. Syphilis was denied.

TABLE I

SUMMARY OF CASES OF AURICULAR FLUTTER WITH 1:1 RESPONSE. MODIFIED FROM BEDELL'S CHART

NO.	REF.	AUTHOR AND DATE	SEX	COLOR	AGE	CARDIAC DISEASE	ASSOCIATED DISEASES	TYPE OF FLUTTER	A-V RATIOS	DURATION	1:1 RATE	REMARKS
1	8	Mackenzie, 1910-1911	M	W	47	None	Mild typhoid before onset	Estab. and Parox.	1:1, 2:1, 3:1?	11 yr.	290 to 300	Attacks following exertion and defecation; controlled by NH_4 Br. Digitalis restored normal sinus rhythm
2	9	Koplik, 1917	M	W	10	None	None	Estab. ?	1:1, 2:1, 3:1, 3:2	2 mo.	225	Marked congestive failure during 1:1 attacks
3	4	Blackford and Willius, 1918	M	W	45	Heart enlarged	Typhoid when 18 yr. old	Estab. ?	1:1, 2:1	2 yr.	224	Attacks of dyspnea-precordial pain radiating to epigastrium. Incapacitating. Duration ½ hr.
4	4	Blackford and Willius, 1918	F	W	32	None	Tonsillitis-exoph. goiter. Biliary colic	Estab.	1:1, 2:1	Several months	320	During one attack ventricular rate was 320 for nearly 2 hr. Patient walked several blocks
5	10	Scott, 1922	M	W	25	None	Influenzal pneumonia 2 yr. before	Estab.	1:1, 2:1	Few months	272	Attacks on slight exertion. Ended by vagal pressure—lying flat. Digitalis induced sinus rhythm. No flutter for 1½ yr.
6	11	Wedd, 1924	M	W	47	Tympanitic aortic second	None	Estab.	1:1, 2:1	6 wk. ?17 yr.	260	Attacks on exertion or excitement. Similar attacks for one month 17 years before
7	12	Gallavardin, 1924	M	W	47	None	Fibroid pulm. tuberculosis	Estab.	1:1, 2:1, 3:2	5 mo. ?30 yr.	270	Attacks following any effort. Once fell on the street
8	13	MacMillan, 1925	M	W	57	Aneurysm and aortitis	Syphilis	Estab.	1:1, 2:1	3 wk. plus	260	Attacks on mild exertion. Duration of attacks, 3 to 5 min.
9	14	Berard, 1926	F	W	??	Compensated mitr. sten. aort. insuf.	Rheumatism denied	Estab.	1:1, 2:1, 3:1, 4:1	6 mo. ?2-3 yr.	250	Attacks on emotion or slight exercise. Digitalis produced 5:1-7:1 block. No more attacks
10	15	Poynton, 1926	M	W	3½	Heart enlarged	None	Parox.	1:1, 2:1, 3:1	3½ yr.	250	Frequent attacks since birth. Attacks lasted several days

TABLE I—CONT'D

NO.	REF.	AUTHOR AND DATE	SEX	COLOR	AGE	CARDIAC DISEASE	ASSOCIATED DISEASES	TYPE OF FLUTTER	A-V RATIOS	DURATION	1:1 RATE	REMARKS
11	6	Parkinson and Bedford, 1927	M	W	43	Mitr. sten. Aort. insuf.	None	Estab.	1:1, no others stated	5 mo.	254	Attacks for 20 min. to 2 hr. Pain about left nipple and down left arm
12	6	Ibid., 1927	M	W	55	Art.-scler.	Syphilis	?	1:1, ?	Unknown	275	Two attacks with syncope
13	16	Herapath, 1928	M	W	28	?	None	Estab. ?	1:1, 2:1	?7 yr.	264	Invalided out of army. After 5 yr. well. Then 2 attacks of flutter in next 2 yr.
14	16	Herapath, 1928	M	W	17	None	Followed influenza	Estab.	1:1, 2:1	8 mo.	288	Attacks produced by exercise. No EKG. of arrhythmia
15	17	Sprague and White, 1928	M	W	49	Heart slightly enlarged	Migraine	Estab.	1:1, 2:1	5 yr.	240	Attacks on exertion—emotion, min. to hr., sometimes stopped by holding full inspiration. Once fell to floor at onset
16	18	Fuller, 1928	M	W	46	Hypertension	None	Estab.	1:1, 2:1, 3:1	1 yr.	240	Weak spells on exertion or excitement —later during rest
17	5	Bedell, 1933	F	C	40	Heart enlarged	Exophthalmic goiter	Estab.	1:1, 2:1, 3:1	8 mo.	260	Patent foramen ovale found at autopsy
18	5	Bedell, 1933	M	C	55	Rare auric. premat. beat	None	Estab.	1:1, 2:1, 3:1	6 mo.	222	Possible attack 10 yr. before. Attacks on exertion
19	00	Carter and Traut, 1934	M	W	60	Followed influenza	None	Estab.	1:1, 2:1, 3:1	1 yr.	200	Vagus effect stopped attacks. Quinidine controlled flutter

He was well nourished. There was slight cyanosis of the lips and finger nails. Pupils reacted promptly to light and in accommodation. Few remaining teeth in fair condition. Tonsils moderately enlarged. No cervical lymphadenopathy. Thyroid normal. Cervical veins not engorged. Heart slightly enlarged. Lower sternum resonant. Radial pulse slow and irregular. Apical pulse regular, except for an occasional dropped beat. No murmurs or

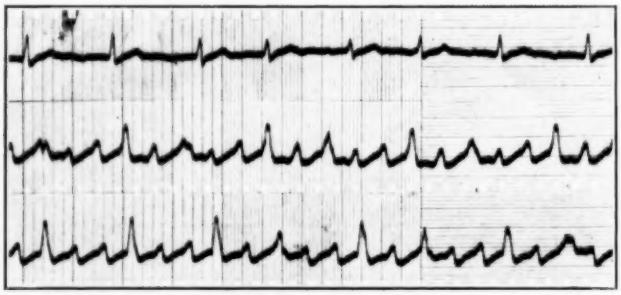

Fig. 1.—Recorded 5/20/33. Before exercise. Auricular flutter with 2:1 and 3:1 ventricular response. Auricular rate 210 per minute. Ventricular rate 80 per minute.

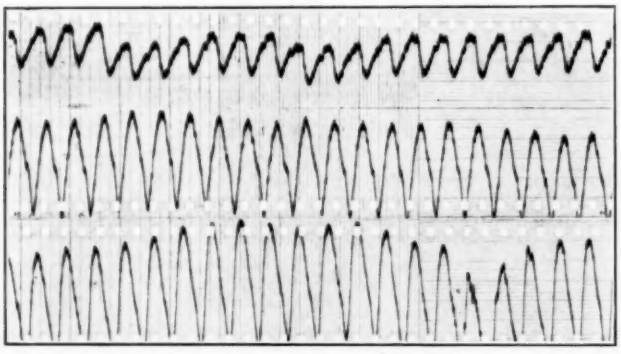

Fig. 2.—Recorded 5/20/33. After rising in bed ten times. Auricular flutter with 1:1 ventricular response. Rate 210 per minute.

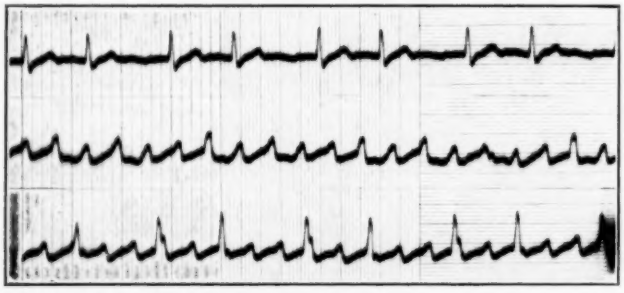

Fig. 3.—Recorded 5/23/33. Before exercise. Similar to Fig. 1.

abnormal accentuations. Blood pressure 128/94. Chest resonant; anteroposterior diameter increased. Lungs clear. Liver and spleen not palpated. No edema. Reflexes normal.

Urine examination: specific gravity of 1.020, alkaline in reaction, and an occasional granular cast. Blood examination, Hb 95 per cent, R.B.C. 5,660,000, and W.B.C. 6,150. Blood Wassermann and Kahn tests were negative. X-ray of chest revealed slight cardiac enlargement.

Progress.—May 18, 1933: Diagnosis was left ventricular hypertrophy due to ancient rheumatism with paroxysmal tachycardia or fibrillation. May 19, patient had attack while straining at stool and again while walking. Rate was 200 per minute and regular. First attack was controlled by continued pressure on supraorbital nerve; the rate changed to 100 and irregular. The second time supraorbital pressure failed to decrease the rate but sitting

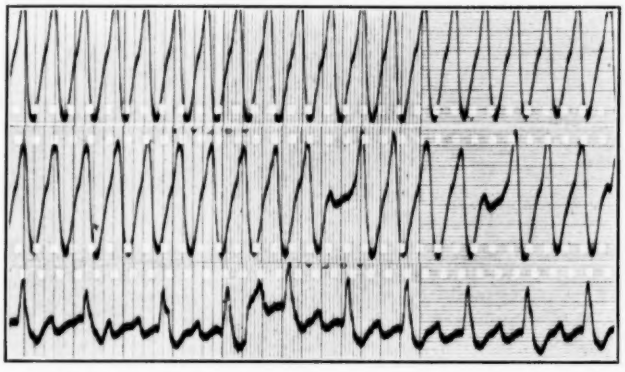

Fig. 4.—Recorded 5/23/33. After exercise. Effect of supraorbital pressure apparent in Lead 2. Cessation of the attack of 1:1 response followed. Flutter with 2:1 response recorded in Lead 3.

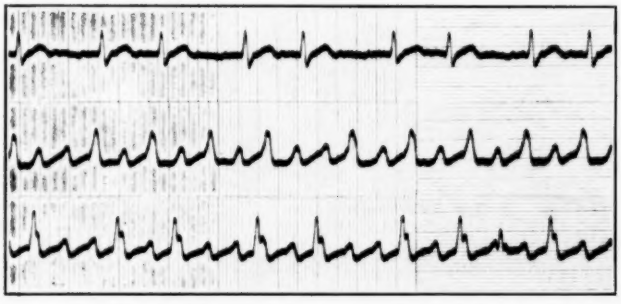

Fig. 5.—Recorded 5/29/33. Before intensive quinidine medication.

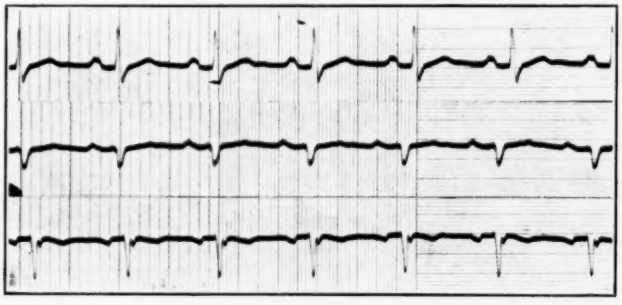

Fig. 6.—Recorded 6/4/33. After intensive quinidine medication.

down relieved the attack. May 20, pulse irregular and slow with alternate strong and weak beats. May 24, frequent attacks of tachycardia. Alternate beats dropped at radial pulse. May 25, attacks more frequent today. Quinidine sulphate gr. iii t. i. d. May 26, no attacks today. May 27, diagnosis of auricular flutter. Quinidine increased to gr. v q. i. d. May 28, definitely improved. Heart regular at a rate of 90 per minute. No attacks since yesterday. May 29, questionable attack yesterday. May 30, no attacks for two days. Pulse

slow and regular. May 31, feels fine. No paroxysms of tachycardia. Still on quinidine gr. v q. i. d. June 1, paroxysm while straining at stool today. Otherwise pulse 60, with coupling of beats at times. June 2, paroxysm on walking. June 3, quinidine gr. v, every hour for 12 doses. June 4, pulse slow and regular. June 6, no paroxysm since intensive quinidine medication, even on walking about the ward. Discharged. To return to cardiac clinic. June 8, walked four miles to cardiac clinic in one and three-quarters hours. Feels fine. Pulse regular. Unable to contact patient since.

COMMENT

Our patient had an established auricular flutter with a suggestive history and characteristic electrocardiographic findings. He responded typically to exercise and to vagus stimulation. Contrary to the experiences of Frey and of Wenckebach and Winterberg,[19] quinidine therapy was not followed by auricular slowing and a paradoxical ventricular tachycardia but by restitution of the normal sinus mechanism.

Because of our previously expressed conviction,[20] quinidine was given continuously. Normal sinus rhythm was maintained.

We are accepting without comment Bedell's analysis of reported instances of auricular flutter with 1:1 response.*

Our records are of paroxysms of 1:1 response, evoked by exercise, influenced by vagus stimulation, preceded and followed by the more typical and orthodox graphs of auricular flutter. Without this evidence, other interpretations of these unusual graphs would be possible.

CONCLUSIONS

Another instance of auricular flutter with a 1:1 ventricular response is added to those previously accepted. In our patient sinus rhythm followed quinidine administration.

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FLUCTUATIONS IN BASOPHILIC AGGREGATION COUNTS WITH METEOROLOGIC ALTERATIONS*

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RED cell basophilia, although not pathognomonic of lead poisoning, has long been associated with this condition and has served as a medium of diagnosis. The presence of basophilic substance has been interpreted as one of the most constant characteristics of immature red cells. McCord (1924, 1928) has devised a method of demonstrating basophilic granules which he has termed the basophilic aggregation test. The basis of this test is the hemolysis of red cells prior to staining with Manson's methylene blue. This results in an artificial aggregation of the evenly distributed basophilic substance found in young red cells, and creates a greater visibility of such cells. McCord states that 66 per cent of normals may exhibit these basophilic aggregations, and he sets the high normal limit at 1.5 per cent in his latest publication. He notes, however, that higher normal counts may occur in the newborn, during pregnancy, after arsenic or iron medications, in high altitudes, after exposure to sunlight and x-rays, and possibly after the intake of foods. Because McCord's normal index was computed on the basis of a single estimate on each of a number of different individuals, the following experiment was devised to see whether one such count was really an indicator of a normal's basophilic aggregation level or whether fluctuations in the level might occur from day to day.

A daily study was made of blood smears from four normal laboratory workers during the month of October, 1934. Subjects I and III were females aged twenty-four years and thirty years. Subjects II and IV were males aged twenty-seven years and twenty-two years. In no instance was there even remotely a lead hazard. Smears were made from Subjects I and II daily except Sundays between the hours of 11:00 A.M. and 12:00 M. Smears were made from Subjects III and IV daily except Sundays between the hours of 2:00 and 3:00 P.M. The smears were stained and examined according to the McCord technic, and the total counts were made on the basis of the number of cells ex-

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Received for publication, May 27, 1935.

hibiting basophilic aggregations per million red blood cells. At the end of thirty-one days the daily counts were charted graphically for each subject. It was immediately noticed that there were daily fluctuations and very definite peaks during the month. It was also noted that in all four cases these peaks coincided rather accurately. Because all of the subjects showed a uniformity in the type of curve, it was felt that some influence common to all had most probably produced this effect. With this in view, the four graphs were compared with a meteorograph for October. The meteorograph has been carried over Figs. 1

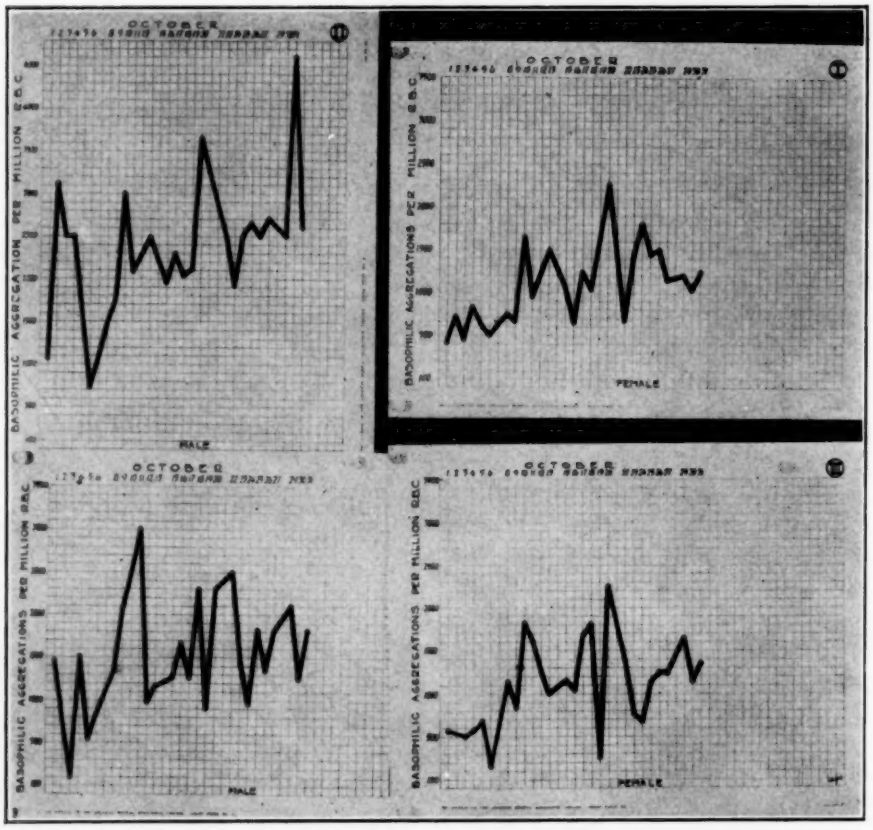

Fig. 1.—This shows the daily fluctuations and the similarity of curves in the four subjects studied.

and 2, and a similar meteorograph over Figs. 3 and 4. In each case stippled vertical columns have been carried from the upper female and lower male basophilic aggregation peaks to the meteorograph above where these have been numbered one to ten. Table I indicates the meteorologic association.

The curves are alike so far as the basophilic aggregation peaks are reached, in general, on the same days and in all cases the increase in young cells is associated with the change to polar air. In Males II and IV no peak is reached on the eighth day of October with episode three as is evidenced in the females, but this effect seems to be cumulative and is carried over to reach the peak on

the tenth and eleventh with episode four. A similar cumulative action is seen in Male IV with episodes nine and ten. The peaks of Subjects II, III, and IV are deferred one day over Subject I in episodes eight and nine. The storm of the twentieth is associated with a marked rise in the curves of all four subjects. In

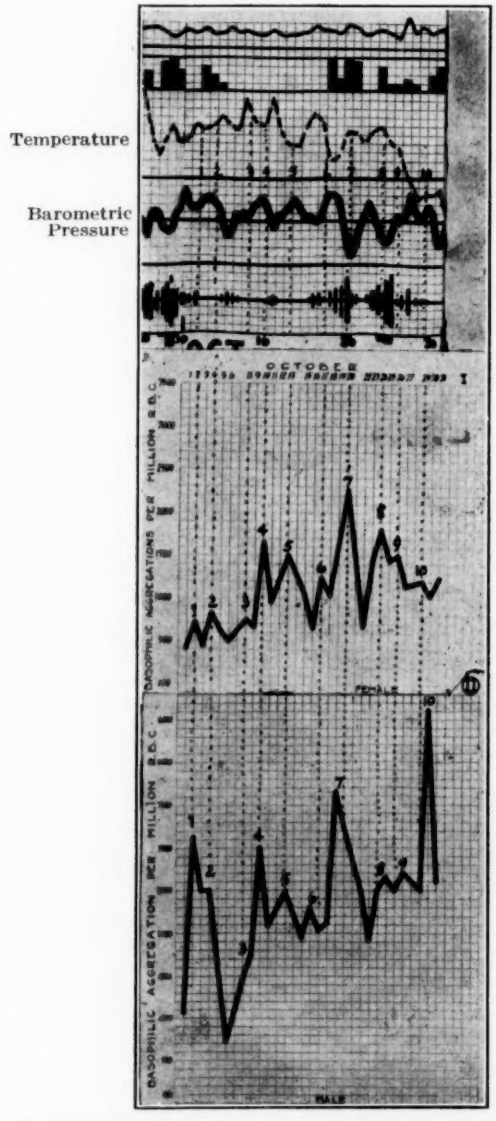

Fig. 2.—This shows how the basophilic aggregation peaks correspond with the high barometric and low temperature peaks in Subjects I and II.

the passage from October to November and the entrance into the more disturbed season of the year, it is seen that the level of the cells exhibiting basophilic aggregations is higher than at the beginning of October. The bone marrow here reflects the effect in a moderate summation in the increased production of young cells.

That blood pressor episodes (ARS phases)* are factors influencing and altering certain cellular and chemical constituents of the blood has been definitely shown by Shulruff, Schiller, Berg, Feinhandler, Milliken and Hoverson. A period of vascular spasm (ARS phase) is followed by (1) an increase in the leucocyte curve; (2) an increase in eosinophile count; (3) an increase in proportionate number of polymorphonuclear leucocytes; (4) the appearance of more young forms; (5) an increase in fibrin; (6) an increase in platelets; (7) an alteration in number of red cells; (8) an increased resistance of red blood cells

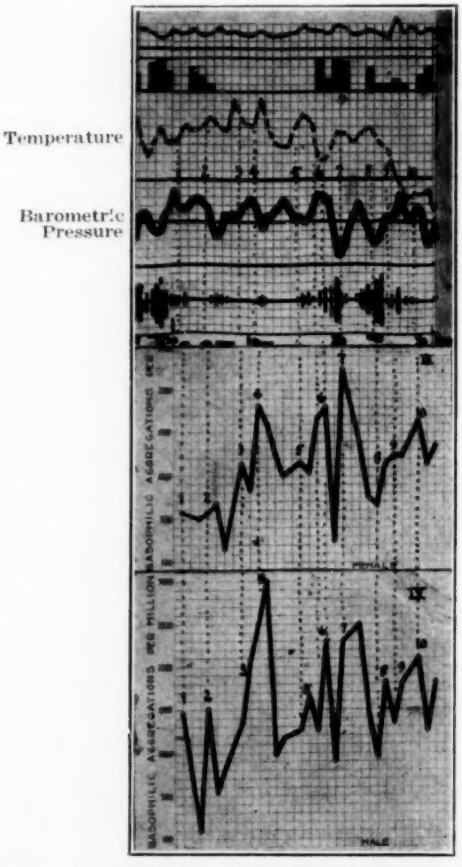

Fig. 3.—This shows how the basophilic aggregation peaks correspond with the high barometric and low temperature peaks in Subjects III and IV.

to saponin and bile; (9) variations in cholesterol content of whole blood and plasma; (10) and an increased sedimentation rate of erythrocytes. Berg also noted an increase in "stippled" cells in the dog during the ARS phase. These

*Petersen has used the abbreviations ARS and COD to designate phase alterations in the normal metabolic status of the individual responding to environmental alterations. In general, the infall of polar air is associated with an increase in the blood pressure, spasm of the vessels, anoxia, and preponderance of reduction. This stage goes over in a continuous flux to the COD phase when tissue metabolism is increased, when oxidation is enhanced, and when the tissue vessels including the capillaries are dilated. The onset of the ARS phase with its spasm of the smaller vessels is promptly associated with the stimulation of the bone marrow due to the relative anoxemia. There follows an increase in leucocytes (young forms as well as eosinophiles), in the red blood corpuscles, platelets, fibrin, etc., and a change in practically all of the serum reactions as well as in the enzymatic and chemical reactions of the body. (See Petersen: Patient and Weather—Autonomic Disintegrations, Ann Arbor, 1934, Edwards Brothers.)

effects may occur synchronously with the peak of the pressor episode or the maximum effect may be delayed until the subsequent barometric low. In Hawaii, where the meteorologic alterations are less intense than in this latitude, Fennel has shown, that there also occurs a definite increase in eosinophiles, polymorphonuclear leucocytes, and total leucocytes, in this instance associated with changes in humidity and wind velocity.

TABLE I

OCTOBER, 1934

EPISODES	DATE OF EPISODES	DATE OF BASOPHILIC AGGREGATION PEAKS				METEOROLOGIC ASSOCIATION
		FEMALE I	MALE II	FEMALE III	MALE IV	
1	2	2	2	1-2	2	Polar infall begins
2	4	4	4	5	4	Polar crest
3	8	7-8	10	8	11	Polar infall
4	10	10	10	10	11	Polar infall
5	13-15	12-13	13	15	13-16	Polar infall
6	17-18	17	16	17-18	18	Polar infall (?)
7	20	20	19	20	20-22	Polar crest on 18th Storm on 20th
8	24	24	25	25	25	Polar infall
9	26	26	27	27	27-29	Polar infall
10	29	29	30	29	29	Polar infall

The results of the above experiments have a rather significant application to the lead industry. In many instances it is compulsory that a basophilic aggregation count or some other standard count be made on new employees, for the purpose of first of all determining whether they have any evidence of previous lead intoxication and second so that subsequent counts made at intervals and compared with the original would indicate whether the individual was experiencing any lead absorption. The making of the initial count or of subsequent counts without giving consideration to normal fluctuations could readily result in misleading information, depending upon whether the count was taken at a polar crest or a polar infall.

SUMMARY

1. Basophilic aggregation counts in normals are subject to rather wide daily variations.

2. In the four normals studied, these variations coincided as is evidenced by the prepared graphs.

3. The only effect in common which could have been experienced by these different individuals would have been an influence due to meteorologic alterations.

4. The daily fluctuations evidenced in these individuals are in accord with the pressor episodes resulting from influx of polar air.

5. Inasmuch as basophilia is an indicator of immature red cells, it can be assumed that an increase in such cells is an evidence of bone marrow stimulation with a liberation of young cells, and in this case is associated with the influence of polar episodes.

6. One basophilic aggregation count does not indicate the normal level for an individual inasmuch as the count may vary widely with the meteorologic changes.

7. In the preliminary basophilic aggregation counts made on individuals entering an industry where there is a lead hazard, the above facts should be taken into consideration in evaluating the results of such counts.

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LYMPHOGRANULOMATOSIS (HODGKIN'S DISEASE)*

A Review of Sixty Cases

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INTRODUCTION

SINCE the original report of Thomas Hodgkin[1] in 1832, there have been many reports of the condition which have been given his name. These have included many surveys, and various aspects of the disease have been emphasized. Because there is still little known as to the etiology and much question about symptomatology and therapy, it was deemed worth while to analyze briefly the sixty proved cases, by biopsy or autopsy, of lymphogranulomatosis admitted to the State of Wisconsin General Hospital during the decade 1924 to 1934. These sixty cases represent 0.00116 per cent of the hospital admissions.

In this study only the pertinent findings, clinical and pathological, will be discussed.

Age and Sex Incidence.—There were forty males and twenty females in all, the ratio being 2:1. All the patients were Caucasians and came from essentially rural districts. Chart 1 illustrates that the relation of the number of males in the various decades of life to the number of females is fairly uniform. The reported age is that at the onset of the disease. However, many of the cases were of as long as one to three years' duration before medical aid was sought. It is of interest that females showed the greatest incidence in the twenty-five- to thirty-four-year period, the males during the thirty-five- to forty-four-year period. No female in this series was less than fourteen years old. There were two males over eighty years of age.

Chief Complaints and Symptoms.—Swelling of the "glands" of the neck, weakness, and cough were the commonest complaints which brought the patient to the hospital. Table I, A, records the chief complaints and Table I, B, the

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Received for publication, May 29, 1935.

most common early symptoms in this series. In many patients there were several complaints; this caused overlapping, and often, difficulty in deciding the chief complaint. Every case had an insidious onset, and these recorded complaints represent the findings after the disease had existed for a considerable period. Every patient complained of weight loss and weakness, and had noted pallor. Most of the patients complained of chills and fever, night sweats, cough, substernal pain, choking spells and anorexia. Symptoms were often dependent upon the nodes involved. Thus enlargement of the cervical, thoracic, abdominal (epigastric), or pelvic groups commonly produced distinctive symptoms as a result of pressure. Not unusually pressure on the cervical sympathetics by neighboring nodes produced inequality in the pupils and pseudoptosis of the eyelid, in three instances true Horner's syndrome. Similarly, pressure by the

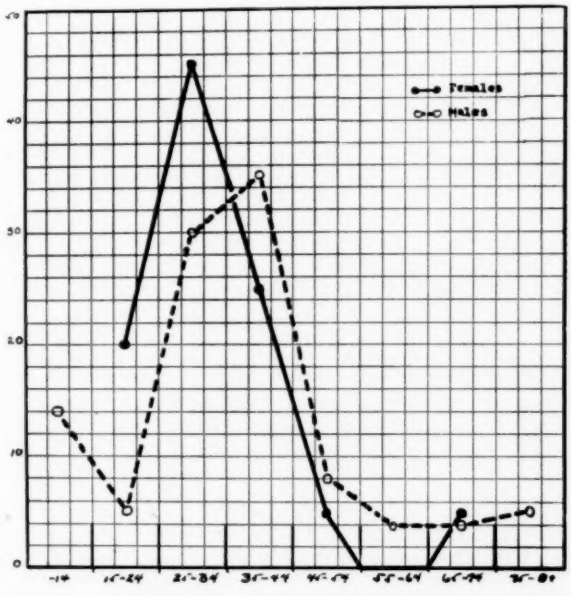

Chart 1.—Percentage of males and females in various age groups for the sixty cases.

intrathoracic nodes produced characteristic symptoms. Nausea, abdominal distress, hydrops of the gallbladder in two instances and other situations resulted from intraabdominal pressure, while symptoms referable to the pelvic organs, such as a feeling of fullness and low back pain, were clearly explained by the involvement of pelvic lymph nodes.

Localization Clinically and at Postmortem.—Although lymphogranulomatosis is a general disease with involvement of the widespread reticuloendothelial system, there are definite areas of localization, which are demonstrable clinically. These can commonly be verified at autopsy. In Table II, Column A is shown the number of cases and percentage of the sixty that clinically showed involvement of certain organs. All organs except the mediastinal nodes were definitely enlarged and palpable. The diagnosis of mediastinal node involvement and bone involvement was usually made by roentgenographic study. In

column B will be found the number of cases and percentage of the sixteen that came to autopsy, and showed gross involvement of organs. In addition there were four cases with only microscopic changes in the liver and spleen. In five cases bone marrow studies were done and disease was found in all. It is noteworthy that mediastinal node involvement was very common, demonstrable in 80 per cent of the 60 cases, and in all of the 16 which came to autopsy.

TABLE I

A. THE CHIEF COMPLAINT THAT BROUGHT SIXTY CASES OF LYMPHOGRANULOMATOSIS TO THE HOSPITAL			B. THE MOST COMMON SYMPTOMS PRIOR TO HOSPITALIZATION		
COMPLAINT	NO. OF CASES	PERCENTAGE	COMMON SYMPTOMS	NO. OF CASES	PERCENTAGE
1. Swelling of the glands of the neck	24	40.8	1. Weakness	60	100.0
2. Weakness	7	11.9	2. Weight loss	60	100.0
3. Cough	6	10.2	3. Cough	50	85.0
4. Lumps in the axilla and the groin	5	8.5	4. Fever and occasional chills	50	85.0
5. Fever	5	8.5	5. Anorexia and occasional nausea	48	81.6
6. Painful glands of the neck with choking sensations	4	6.8	6. Dyspnea and choking spells	40	68.0
7. Nausea and epigastric distress	3	5.1	7. Pain in chest	34	58.8
8. Pain in right leg	3	5.1	8. Night sweats	31	52.7
9. Backache	3	5.1	9. Nocturia (1-3x)	24	40.8
			10. Backache	20	33.3
			11. Edema	18	30.6
			12. Headaches, tinnitus, and vertigo	15	25.5

TABLE II

NUMBER OF CASES THAT SHOWED GROSS INVOLVEMENT OF CERTAIN ORGANS

	A. CLINICAL INVOLVEMENT		B. POSTMORTEM	
ORGAN	NO. OF CASES	PERCENTAGE	NO. OF CASES	PERCENTAGE
Superficial nodes	58	96	14	88
Mediastinal nodes	50	85	16	100
Abdominal nodes	20	33.3	13	81
Spleen	40	66.6	12	75
Liver	55	93	12	75
Pelvic nodes	3	5	11	69
Bone (roentgenogram)	9	15	5 (micro)	31

At autopsy, the liver was grossly involved as often as the spleen, and on clinical examination the liver was palpable more often than the spleen. The palpability of the spleen in two-thirds of the cases is about the frequency usually noted in any series.

Involvement of the Special Organs Including the Skin.—In certain cases the disease was found at sites not so commonly involved. These instances were confirmed by biopsy or autopsy. There were 8 cases with infiltration into the lung tissue, 7 of these with an associated atelectasis, 3 with pleural involvement, and 2 with erosion of the bronchi. Three of the cases showed gastric and 3 pericardial involvement. The myocardium and the superior vena cava were each involved in 2 cases. There was 1 example of infiltration into each of the following organs: Thymus, sternum, right auricle of the heart, pectoral muscle, kidney, urethra, bladder, and 1 case with multiple ulcerat-

ing areas in the colon. Involvement of many of these organs could be diagnosed only at autopsy: therefore the actual incidence in the entire series cannot be stated.

Gross skin manifestations were seen in 11 patients. There were, however, 14 additional patients who had pruritis without visible skin changes. These lesions were most commonly found on the exposed surfaces of the extremities and were characterized by an atrophic eczematoid chronic eruption often simulating a fungous growth. The margins usually had traumatic abrasions from scratching. Two cases showed ulceration of the skin over enlarged nodes. Increased pigmentation was found in 27 cases. This was usually characterized by dark brown, molelike, flat, blotchy areas on exposed surfaces of the body, but a general bronzing occurred in 4 patients. Edema was usually related to lymph stasis or vascular obstruction; much less often cardiac decompensation was the cause.

Temperature, Pulse, and Blood Pressure.—After the disease had established itself and was active, there was fever. This was usually characterized by a remittent temperature elevation with an evening rise of two to three degrees. The average duration of the pyrexia was found to be three weeks in 54 cases and usually occurred in the latter half of the illness. The fever was usually irregular, simulating various diseases as undulant fever (2 cases), and typhoid (3 cases). Pel-Ebstein fever occurred in 1 case. The 4 patients with leucopenia usually had a subnormal morning temperature and a minimum evening temperature of 103° F. There were no patients with a continuous subnormal temperature.

The pulse was found to be rapid in every patient after the disease was well established. The rate usually varied between 100 and 120 per minute. There was a definite increase in pulse rate concomitant with the evening fever. Those patients who had an increased basal metabolic rate also had a simultaneously increased pulse rate.

Hypotension was the rule. The hypotension was not related to the extensiveness of the lesions or to the height of the daily temperature. The average systolic pressure was 110 and the diastolic pressure 74 mm. Hg. When an elevated blood pressure did exist, arteriosclerosis of considerable degree was present. Only one patient had marked hypertension.

Laboratory Findings.—As yet there is no specific diagnostic laboratory test for lymphogranulomatosis other than microscopic study of the biopsy tissue, though the test of Gordon,[2] recently described, may be useful. The characteristic microscopic picture on section is well known; the occasional diagnostic difficulties in study of the biopsy tissue are also well recognized, and it is not our purpose to discuss these phases.

Aside from the examination of an excised node the blood findings have received the most attention. The characteristic changes in the leucocyte formula have been emphasized by Bunting.[3] In these 60 cases the blood findings were not absolutely uniform. In Charts 2 and 3 will be found the blood findings in percentage of cases on admission, the average during residence in the hospital, after x-ray therapy, and the last count before death. More than two-thirds of the cases showed a leucocyte count above 8,000 and a majority

were between 10,000 and 25,000. The percentage of neutrophiles was distinctly above normal and as the disease progressed, there was a progressive decrease in lymphocytes. The leucocytosis was not always greatest in those cases with highest fever; in fact those cases with a marked septic type of fever showed a leucopenia. There was a definite increase in the percentage of large mononuclear cells as the disease progressed. Only 5 per cent of the cases showed an eosinophilia above 5 per cent. Twenty-four (40 per cent) of the cases showed immature forms of erythrocytes. The anemia was usually moderate, the erythrocyte count being over 3,000,000 and the hemoglobin

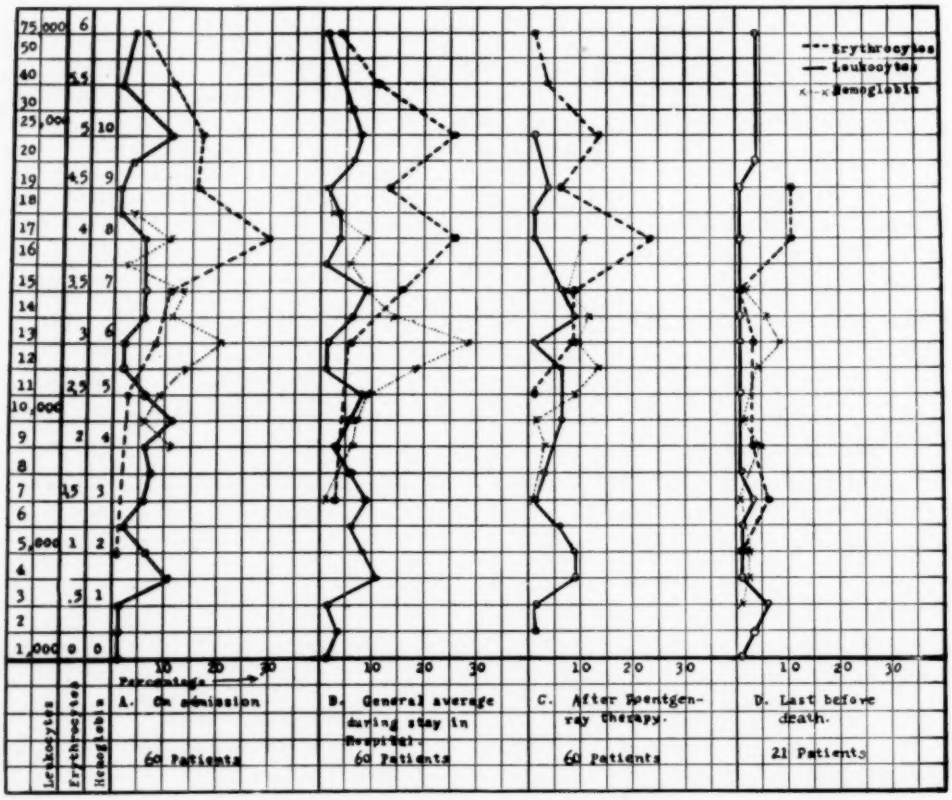

Chart 2.—The number of leucocytes, erythrocytes, and the hemoglobin at various periods of hospitalization of the sixty cases.

usually 55 to 60 per cent of normal. Of the 16 cases in which platelet counts were studied only 6 showed a moderate decrease as a deviation from the normal. All of the patients had a negative Wassermann.

The basal metabolic rate is of little value from a diagnostic standpoint but it has been noted (Krantz)[4] that there was a definite increase in the so-called active cases. Of the 31 cases in which the metabolic rates were determined 22 showed a rate above +10 per cent. The average of the first tests for the 22 cases was +23.2 per cent.

Concerning Early Diagnosis.—An earlier diagnosis of lymphogranulomatosis is desirable, but this is frequently fraught with difficulty because of the in-

sidious onset without characteristic subjective symptoms. The common general complaints of weakness, easy fatigue, and weight loss are too common to permit suspicion of the specific disease. The additional common complaint of excessive perspiration, undoubtedly associated with an increased metabolic rate, has probably been a factor in leading to the occasional mistaken diagnosis of thyrotoxicosis.

Nearly every patient complained of cough of more or less degree, without gross evidence of pulmonary disease. The possibility of mediastinal node involvement as the cause of this symptom might suggest itself since it is very common. In several of these patients the cough was ignored or apparently explained by a goiter.

In the last analysis, the most important complaint, which is a common one that might often lead to an earlier diagnosis, is that of an enlarged node, and

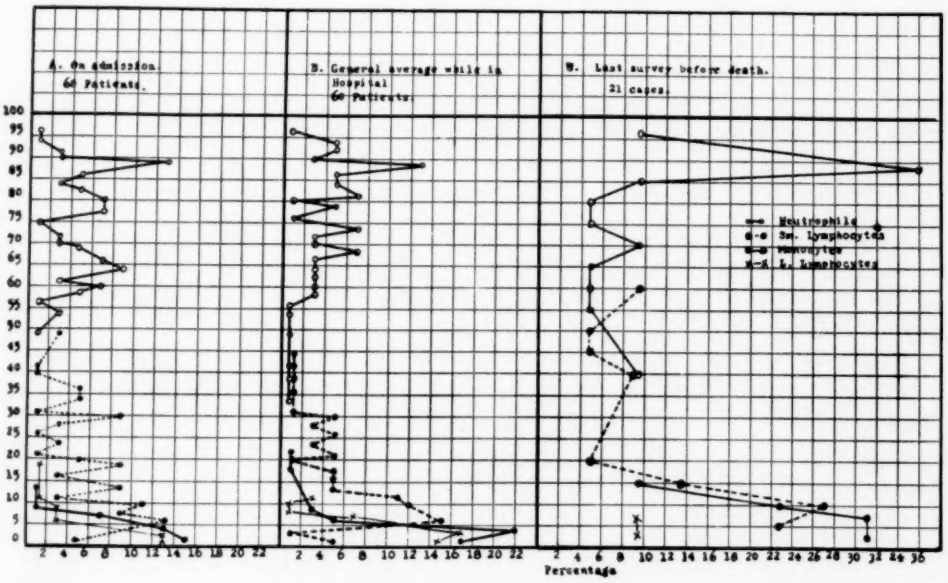

Chart 3.—The percentage of neutrophiles, large and small lymphocytes, and monocytes at various periods of hospitalization of the sixty cases.

if early removal and microscopic examination is practiced, comparatively early diagnoses will be made. Of course, occasionally there will be no node available for biopsy.

Roentgenograms are of distinct value, especially in the detection of mediastinal involvement, in finding the cause for certain bizarre symptoms due to obstruction of a hollow viscus, and for discernment of bone involvement. Roentgenograms of the chest in these cases should be a matter of routine because of the regular involvement of the mediastinal nodes, in 100 per cent of the sixteen cases of this series that came to autopsy. Roentgenographic examination of the bones is indicated when referable complaints exist, especially those of the back since involvement of the spine is far from rare.

Prognosis.—Because of the nature of the service at the Wisconsin General Hospital, where patients are referred by local physicians from various parts

of the state and then discharged to return to the care of their physicians, adequate follow-up observation is not possible. Consequently our figures as to duration of life with the disease cannot be highly accurate and no attempt is made to arrive at a prognostic figure from the cases of this series. From the patients who died in the hospital, however, it is possible to conclude that the average duration of the disease is approximately that which has been generally accepted, two to three years. Nearly every clinic has, however, observed patients who live much longer than is usual and that was true in this series. Two patients have now been under observation for more than twelve years. The diagnosis in both instances was made from biopsy of a lymph node by Dr. C. H. Bunting.

Treatment.—It is not within the scope of this paper to discuss therapeutic measures and results of therapy in detail. The treatment in all cases has taken into account the general hygienic measures and the treatment of symptoms as they arose. In recent years an especial attempt has been made to enforce the intake of a high caloric diet with a minimum of 3,000 calories, and there is a distinct impression that as a result the patients have done better than with the ordinary diet. The better results are not obtainable, however, unless a real attempt is made to cater to the patients' likes and dislikes, and to enthusiastically stimulate their interest in an abundance of food.

High voltage (180 K.V.) roentgen ray therapy has been a mainstay in most cases. All patients received it where there was no contraindication, i.e., grave uncontrolled leucopenia or anemia, a state of extremis on admission or some other contraindicating situation. In all cases, the therapy was directed to the involved sites such as the enlarged lymph nodes, the spleen, the liver, or the vertebrae. Early in the disease, satisfactory subjective and objective response to this therapy was the rule. Gain in weight and strength usually ensued, there was decrease in the size of the involved lymph nodes, spleen, and liver. This improvement was always temporary, but for variable periods of time. The lymph node enlargement recurred and the evidences of extension of the disease became more prominent. Ultimately there was no response to therapy, the lymph nodes no longer resolved, and the subsequent duration of life was invariably short. The results in this series from roentgen ray therapy tended to confirm those of Minot and Isaacs,[5] and it seems possible to conclude as they did, that roentgen therapy makes for greater comfort for these patients, and in some cases, by relief of mechanical obstruction, considerable prolongation of life. This series is too small, and particularly is that true of the unirradiated group, to permit any conclusion as to the effect of roentgen ray therapy upon longevity in the average case.

COMMENT

Analysis of these sixty cases confirms in large part many of the established facts regarding Hodgkin's disease. The prevalence of the disease in the middle and early age groups, and the two to one predominance of the disease in males were to be expected. The high incidence of fever and tachycardia, and the frequence of an elevated basal metabolic rate are all commonly accepted as part of the disease. Likewise, the studies of Bunting,

showing the usual existence of a moderate leucocytosis with a persistent increase in large mononuclear cells and a progressive neutrophilia with a concomitant progressive lymphopenia in Hodgkin's disease, are confirmed by the majority of these cases. These findings in the differential blood picture are not universally acknowledged; nevertheless, from this series one must agree with Bunting's findings. The detail of eosinophilia in the second period of the disease was not, however, uniformly observed in these cases. This may perhaps be explained by the fact that many of the cases came to us comparatively late in their course. The great frequency of immature erythrocytes in increased numbers in the peripheral blood is noteworthy.

Analysis of the chief complaints offered by these patients brings out nothing new or striking. As might be anticipated, the chief complaint of "swelling of the glands of the neck" was the most common. Of the other complaints, however, those suggesting intrathoracic disease were very frequent. Cough, dyspnea and choking spells, chest pain, and night sweats occurred in more than half of the cases, cough being the most frequent, occurring in 83 per cent. The great frequency of these symptoms is not generally appreciated, and they tend to confirm the surprisingly high incidence of mediastinal lymphadenopathy in these sixty patients. Too much emphasis cannot be placed upon the importance of roentgenograms of the chest in all cases of Hodgkin's disease because of this high incidence and the great difficulty in establishing the existence of mediastinal involvement by physical examination alone.

The occasional unusual sites for the existence of Hodgkin's lesions in these cases emphasizes the well-known fact that practically any organ of the body may become involved.

SUMMARY

Sixty cases of Hodgkin's disease, sixteen of which came to autopsy, have been observed and an analysis has been made of the commoner subjective and objective clinical findings and the postmortem lesions. Signs referable to the respiratory system and the finding of mediastinal lymphadenopathy were surprisingly common. Hypotension, fever at some time in the disease, tachycardia and an increase in the basal metabolic rate were frequently noted. The blood findings were similar to those described by Bunting as characteristic of the disease. Marked relative and absolute lymphopenia were common as the disease progressed.

High voltage roentgen therapy relieved symptoms in most of the treated cases. This series did not permit conclusions relative to prolongation of life as a result of irradiation except in rare instances when the therapy brought about diminution in the size of nodes that were producing mechanical obstruction which appeared to be incompatible with life.

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THE RESPONSE OF BLOOD UREA NITROGEN, URIC ACID AND PLASMA CHOLESTEROL TO PARENTERAL LIVER EXTRACT*

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DURING the course of diffuse glomerulonephritis one finds renal decompensation, either temporary or permanent. In the progression of events (see Chart 1) such occasions of decompensation (azotemia) are observed in the acute stage, also in the severe subacute type and in the end stage. Azotemia may occur also when an acute exacerbation is superimposed on chronic nephritis and when chronic nephritis is complicated by an acute infection or cardiac decompensation.

The treatment of diffuse glomerulonephritis is as yet symptomatic, and will probably remain so until the causative agent is discovered. When the kidneys fail to rid the body of the end-products of protein metabolism, other organs assist in excretion. Thus the uriniferous breath, the uremic frost, the diarrhea are all signs of the attempt by the salivary glands, the skin and the bowel, respectively, to help out in this emergency. Pucher and Sly[13] found an increase in urea nitrogen in the gallbladder bile of patients dying with urea nitrogen retention in the blood. They studied patients with pneumonia, peritonitis and nephritis in whom there was an increase in blood urea nitrogen. They observed that the composition of the blood has a pronounced effect on the amounts of the nonprotein nitrogen constituents found in gallbladder bile.

Our Problem.—Using concentrated liver extract parenterally, we desired to promote extrarenal excretion of urea nitrogen. A stimulant that would increase the liver's excretory power has a place in the treatment of azotemia. Dr. Richard Bauer,[2] Professor of Medicine, University of Vienna, reported on the use of parenteral liver extract in treatment of coma due to liver insufficiency. Perhaps parenteral liver may augment the liver's excretory capacity. We studied the effect of parenteral liver on blood urea nitrogen, uric acid, and plasma cholesterol. The latter was included because of clinical evidence of a reciprocal relation between the blood urea and plasma cholesterol.

It is common knowledge that as chronic nephritis progresses toward the end stage, cholesterol diminishes as the urea nitrogen rises (see Chart 1). As more and more glomeruli become completely obliterated by disease, they no longer are the site at which proteins are lost from the blood into the urine. The defective filtration area is greatly reduced, protein loss is diminished, and the blood proteins gradually tend to return to normal. With this increase in blood

*From the Medical Services of the Jewish Hospital of Philadelphia.
Received for publication, June 4, 1935.
Thesis submitted to the Faculty of the Graduate School of Medicine of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Master of Medical Science (M. Sc. (Med.)) for graduate work in Internal Medicine.

proteins (oncotic pressure), plasma cholesterol decreases. Bruger and Poindexter[5] observed a diminution of plasma cholesterol upon the ingestion of urea. They considered this result as possibly due to increased osmotic pressure exerted by the urea.

As urea increases in the blood, urea excretion by the liver increases.[13] Perhaps this mode of extrarenal urea elimination brings about a concomitant excretion of plasma cholesterol by the bile. Having this possibility in mind, we studied the effect of parenteral liver on urea nitrogen and cholesterol.

Selection of Patients.—The studies were carried out on patients in the wards of the Jewish Hospital. The patients, white males and females, ranged in age

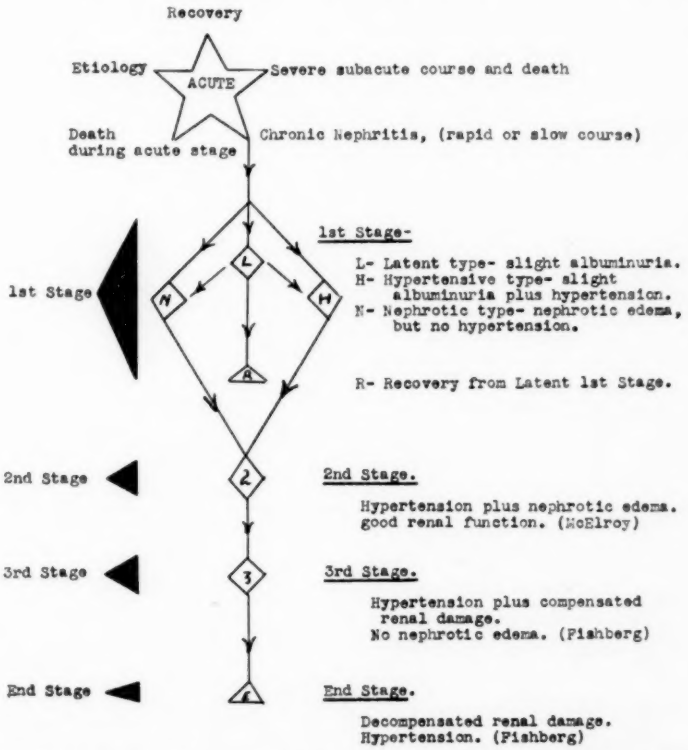

Chart 1.—Diagram of the succession of events in diffuse glomerulonephritis. Bright's disease may make its first appearance clinically as chronic diffuse glomerulonephritis, the patient having been unaware of an acute attack.

between twenty-four and seventy-five. Each one selected had been in the wards at least three days prior to the beginning of studies. Patients with biliary obstruction were excluded. Hawkins and Wright[9] have observed hypercholesteremia in such cases. Tindal[15] observed a prolonged blood nonprotein nitrogen curve upon feeding urea to children suffering from various forms of hepatic disease. Patients suspected of having hepatic disease were therefore not included.

Method of Study.—The first fourteen patients, Group A, were studied as follows. First day: at 8:30 A.M. blood for urea nitrogen, uric acid, plasma cholesterol determinations; at 10:00 A.M. 6 c.c. concentrated liver extract (Lilly or Lederle G fraction of Cohn) intramuscularly; at 2:30 P.M. one-half ounce

of a 50 per cent solution of magnesium sulphate. Second day: at 6:30 A.M. one-half ounce of magnesium sulphate; at 8:30 A.M. blood for urea nitrogen, uric acid, and plasma cholesterol; at 10:00 A.M. 6 c.c. liver extract intramuscularly; at 2:30 P.M. magnesium sulphate. Third day: at 6:30 A.M. magnesium

TABLE I

GROUP A. LIVER EXTRACT PLUS MAGNESIUM SULPHATE

PATIENT	DIAGNOSIS	DAY	UREA N. MG.	URIC ACID MG.	CHOLESTEROL MG.	ALBUMIN GM.	GLOBULIN GM.
1	Chronic nephritis	First	121	13.7	312	2.9	0.9
		Second	114	15.6	314		
		Third	127	12.5	312		
2	Chronic nephritis	First	65	8.6	227	3.3	1.7
		Second	60	8.4	238		
		Third	69	8.9	200		
3	Chronic nephritis	First	61	10.7	290	3.2	1.1
		Second	60	11.7	337		
		Third	58	11.0	294		
4	Nephrosclerosis	First	54	7.5	263	5.2	1.7
		Second	55	10.0	200		
		Third	55	8.7	166		
5	Chronic nephritis	First	41	8.2	196	4.6	1.4
		Second	41	8.7	150		
		Third	41	8.5	192		
6	Nephrosclerosis	First	49	4.8	156	4.2	2.1
		Second	40	6.0	130		
		Third	35	5.0	135		
7	Nephrosclerosis	First	20	3.5	140	4.6	2.4
		Second	23	6.0	139		
		Third	23	4.2	140		
8	Nephrosclerosis	First	17	2.6	208	5.1	1.8
		Second	10	2.1	166		
		Third	20	3.0	165		
9	Diabetes	First	17	5.0	166	5.1	2.0
		Second	16	4.8	207		
		Third	18	5.2	277		
10	Diabetes	First	28	8.0	200	4.0	1.2
		Second	23	9.0	243		
		Third	20	8.2	227		
11	Diabetes	First	23	4.6	279	5.1	1.3
		Second	20	3.7	227		
		Third	17	5.2	249		
12	Diabetes. Pituitary dysfunction	First	18	5.2	147	5.5	0.5
		Second	23	4.7	138		
		Third	20	4.1	140		
13	Diabetes	First	26	3.0	208	4.6	1.9
		Second	16	3.2	156		
		Third	18	3.2	192		
14	Diabetes	First	18	3.7	238	4.2	2.8
		Second	18	3.8	192		
		Third	15	3.5	178		

sulphate; at 8:30 A.M. blood for urea nitrogen, uric acid, and plasma cholesterol. The magnesium sulphate was given to promote gallbladder emptying. The patients experienced about three bowel movements a day.

The next ten patients, Group B, were studied for the effect of liver extract on cholesterol alone. These did not receive magnesium sulphate or any other drastic cathartic. Blood was collected for plasma cholesterol determinations on three consecutive days at 8:30 A.M. At ten o'clock of the first and second mornings the patients received 6 c.c. of liver extract intramuscularly.

Group C consists of two patients who received 3 c.c. of liver extract intramuscularly for twelve successive days. Plasma cholesterol determinations were made before and after.

In Group D are three patients who received magnesium sulphate alone. The time of administration of the salt and collection of blood for plasma cholesterol was the same as that in Group A.

TABLE II

GROUP B. LIVER EXTRACT—CHOLESTEROL

PATIENT	DIAGNOSIS	DAY	CHOLESTEROL MG.	ALBUMIN GM.	GLOBULIN GM.	SUGAR MG.	DAILY INSULIN
1	Diabetes	First	294	4.5	2.5	192	
		Second	217				
		Third	300				
2	Diabetes	First	192	4.6	1.4	168	45
		Second	200				
		Third	208				
3	Diabetes	First	263	4.1	1.9	206	40
		Second	200				
		Third	217				
4	Diabetes	First	172	4.4	1.6	174	50
		Second	200				
		Third	192				
5	Diabetes	First	249	4.2	2.8	150	30
		Second	192				
		Third	208				
6	Nephrosclerosis	First	186	5.6	1.2	86	
		Second	263				
		Third	249				
7	Nephrosclerosis	First	178	6.5	1.9	81	
		Second	192				
		Third	178				
8	Chronic nephritis	First	227	3.3	1.7	118	
		Second	230				
		Third	200				
9	Parkinsonism	First	156	5.6	5.6	114	
		Second	166				
		Third	157				
10	Convalescent pneumonia	First	189	6.5	1.7	130	
		Second	172				
		Third	178				

The blood cholesterol of three patients, Group E, were studied on three consecutive mornings. These received neither liver extract nor magnesium sulphate.

Such drugs as digitalis, insulin, and phenobarbital which the patients received before the experiment was begun, were allowed during the experiment. No drug was introduced during the period of study. Serum albumin-globulin determinations were made on all the patients. All blood studies were performed on fasting subjects by the same technician in the Laboratories of the Jewish Hospital. The following methods were used: Marshall's Modified Aeration for urea nitrogen, Folin-Wu for uric acid, Bloor for cholesterol, Folin-Wu and Miero-Kjeldahl for serum albumin and globulin. The blood chemistry quantities are recorded for 100 c.c. of blood.

TABLE III

GROUP C. LIVER EXTRACT, 3 C.C. DAILY FOR TWELVE DAYS

PATIENT	DIAGNOSIS	DAY	CHOLESTEROL MG.	ALBUMIN GM.	GLOBULIN GM.	SUGAR MG.	DAILY INSULIN
1	Pernicious anemia	First	198	4.6	1.2	98	
		Thirteenth	149				
2	Anemia; diabetes	First	248	5.1	1.3	196	55
		Thirteenth	210				

TABLE IV

GROUP D. MAGNESIUM SULPHATE

PATIENT	DIAGNOSIS	DAY	UREA N. MG.	URIC ACID MG.	CHOLESTEROL MG.	ALBUMIN GM.	GLOBULIN GM.
1	Diabetes	First	23	3.0	178	4.6	1.9
		Second	20	4.5	217		
		Third	29	3.7	208		
2	Diabetes	First	20	3.8	238	4.2	2.8
		Second	23	5.7	192		
		Third	23	5.5	227		
3	Nephrosclerosis	First	16	4.1	198	5.5	1.2
		Second	15	3.4	192		
		Third	18	4.1	217		

TABLE V

GROUP E. NO MEDICATION

PATIENT	DIAGNOSIS	DAY	CHOLESTEROL MG.	ALBUMIN GM.	GLOBULIN GM.	SUGAR MG.	DAILY INSULIN
1	Diabetes	First	217	4.1	1.9	200	40
		Second	190				
		Third	180				
2	Chronic nephritis	First	300	3.3	1.7	118	
		Second	273				
		Third	249				
3	Diabetes	First	277	4.2	2.8	138	35
		Second	279				
		Third	247				

COMMENTS AND OBSERVATIONS

Each patient was on practically the same diet before and during the experiment; rigid control of diet was not attempted. Bruger and Poindexter[3, 5] observed no immediate change in plasma cholesterol values following the ingestion of water and fat. No immediate response in plasma cholesterol was observed by Bruger and Mosenthal[4] following the injection of insulin and epinephrine. Bruger and Somach[6] obtained a daily variation of 8 per cent plus or minus in the plasma cholesterol of normal subjects. In our three patients (Group E) the following variations were obtained: in the first patient, plus 14 per cent and minus 5 per cent; in the second, plus 9 per cent and minus 8.8 per cent; and in the third, plus 0 per cent and minus 10 per cent.

Gebhardt and Klein[7] observed an increase in plasma cholesterol of nineteen patients following intramuscular injections of liver extract. They administered 3 c.c. of the extract daily for a period of eight to twenty-two days. The increase was occasionally as high as 50 mg. In our two patients, Group C, we observed a decrease of 49 mg. in one, and 38 mg. in the other.

In studying the response of plasma cholesterol to liver extract in Groups A and B, one finds no consistent results if he considers all patients alike. However if the diabetic patients are separated from the nephritic patients and the nephrosclerotic patients, one observes the following: In all the diabetic patients of Groups A and B, whose plasma cholesterol was above 200 mg. on the first day, a decrease is seen on the second day (initial fall). Thus in Cases 11, 13, and 14 of A the plasma cholesterol diminished 18.5 per cent, 25 per cent, and 19 per cent, respectively, following the first injection. Cases 1, 3, and 5 of B had a decrease of 22 per cent, 23 per cent, and 23 per cent, respectively. In all the other diabetic patients the plasma cholesterol on the first day was 200 mg. or less. All of these, except Case 12A, showed a rise in cholesterol. (Case 12A is one of pituitary dysfunction; the diabetes may be secondary to the pituitary disturbance.) The increase in cholesterol was 4 per cent, 16 per cent, and 26 per cent in Cases 9A, 2, and 4 of B, respectively.

Among the nephrosclerotic patients there were two patients whose plasma cholesterol on the first day was above 200 mg. Both of these showed a fall in cholesterol following the first injection of liver (Cases 4 and 8 in A). However only one case (6B) of the remaining nephrosclerotic patients whose plasma cholesterol was 200 mg. or less revealed a rise. In this respect the response in the nephrosclerotic patients differed from that of the diabetic patients.

In some of the cases which showed a decrease of plasma cholesterol following the first injection, a slight rise was noted following the second. This however was not uniform.

The response of cholesterol in diabetic patients to liver alone and to liver plus magnesium sulphate is apparently the same as that to magnesium sulphate alone. In Group D (response to magnesium sulphate) there are two diabetic patients, one with a cholesterol above 200 mg. These two behaved exactly as the diabetic patients in the other groups. The one with 178 mg. increased to 217 mg. (22 per cent), the one with 238 mg. decreased to 192 mg. (19 per cent).

The serum protein level is probably one of the factors concerned with the

response of cholesterol to liver and to magnesium sulphate. Four of the five nephritic patients had a low total serum protein (Cases 1, 2, 3 in A and 8B). The initial fall in cholesterol was not observed in these although the cholesterol was above 200 mg. While in Case 5A with a total serum protein of 6 gm. the cholesterol decreased from 196 mg. to 150 mg. (23 per cent).

The response of blood urea nitrogen and uric acid to liver and magnesium sulphate was neither constant nor significant.

SUMMARY AND CONCLUSIONS

1. The effect of parenteral liver extract on blood urea nitrogen, uric acid, and plasma cholesterol was studied.

2. No significant response in urea nitrogen and uric acid was observed.

3. The response of plasma cholesterol in the diabetic patients was uniform. (a) Diabetic patients with plasma cholesterol *above* 200 mg. showed a *decrease* following the first injection. (b) Diabetic patients with plasma cholesterol of 200 mg. or less showed an *increase* following the first injection.

4. The change (initial rise and fall) in the plasma cholesterol level obtained with parenteral liver alone is apparently the same as that obtained with magnesium sulphate alone.

5. Serum protein is one of the factors governing the response of plasma cholesterol to parenteral liver.

6. We did not observe an increase in plasma cholesterol following liver injections daily for twelve days as had been previously reported by another writer.[7]

We are most appreciative for the cooperation of the Jewish Hospital and the Jewish Hospital medical staff. We are indebted to Dr. Edward W. Steinfield and Mrs. Carrie B. Nash for their help in the blood chemistry studies.

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AUTOHEMAGGLUTININ*

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THERE is a lack of appreciation of the fact that autohemagglutinin is not of uncommon occurrence in human blood, and that it almost surely has a definite relation to posttransfusion reactions. The following case is instructive.

The patient was a girl of nine years in the twelfth day of a typical attack of lobar pneumonia. On this day an extension to an additional lobe had occurred. No sputum could be obtained for bacteriologic study. Blood culture had been sterile. Transfusion was recommended.

The patient was blood Type A. *Clot* of both patient and an A donor were warmed to 37° C. just before separation of the serum. Cells were washed twice in warm saline. On microslides the patient's serum was found to agglutinate her own cells, and those of the donor almost completely in thirty minutes at 20° C. and at 9° C. The same combinations of serum and cells placed at 37° C. for thirty minutes in a moist chamber showed no agglutination at all. The patient's serum likewise agglutinated the cells of an 0 blood (unwashed) at 20° C. The donor's serum showed no agglutination in any case.

Five-tenths of a cubic centimeter of the patient's serum and 0.5 c.c. of the patient's packed washed cells were placed at 2° C. for thirty minutes and shaken several times. The tube was centrifuged while surrounded with ice water, and the serum removed at once. This serum showed only a trace of agglutination of the patient's cells, the Type A donor's cells and Type O cells at 9° C., after thirty minutes. The agglutinin had been absorbed almost completely.

The patient's serum, unheated, was progressively diluted and mixed with equal amounts (0.5 c.c.) of a 1 per cent suspension of the patient's cells and of the cells of the Type A donor. After thirty minutes at 9° C., agglutination was visible to the eye in dilutions up to and including 1:16 in both series. Drops removed and observed with the microscope showed agglutination up to and including dilution 1:64. These same tubes were then placed at 37° C. for thirty minutes, after which all agglutination had disappeared as observed with the microscope. There was no hemolysis.

The clumping produced by the patient's serum when mixed with her own and with the donor's cells had an appearance typical of iso-agglutination. A few rouleaux, however, were seen. To both these mixtures lecithin as a rather heavy saline suspension was added. After thirty minutes at 20° C. there was disappearance of the rouleaux, but only slight breaking up of the clumps of cells. The majority of the clumps persisted.

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Received for publication, June 27, 1935.

This blood, then, contained an autohemagglutinin of 1:64 titer, typical in that it was sensitive to changes in temperature, reversible, absorbable, and not appreciably affected by lecithin.

Transfusion was not attempted.

The autohemagglutinin, unless of extremely high titer, is not detected by the customary technic, which makes use of chilled clots and room temperature incubation of the serum-cell mixtures.

TIMES MEDICAL BUILDING

MEDICAL ASPECTS OF PERIODONTOCLASIA AND GINGIVITIS*

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PERIODONTOCLASIA—pyorrhea alveolaris, interstitial gingivitis, or Rigg's disease—is one of the oldest oral diseases peculiar to man. Its incidence has been noted in skulls reputed to be more than thirty thousand years old.

In the second book of Hippocrates there is a description of an oral condition in which the gums are unhealthy and the teeth loosened. The objective findings of Hippocrates coincide with the signs of our present-day prevalent pyorrhea. In modern times medicine and dentistry as professions have recognized the universality of the disease and have instituted a vast amount of laboratory and clinical research concerning its pathogenesis and therapeutics.

The literature of this subject is extensive and conflicting. Contradictory definitions and classifications render the majority of the articles almost valueless when they are studied critically. From an internist's viewpoint, and in view of the material to be presented in this communication, the most satisfactory definition found was that of Delater:[1] "Pyorrhea is a chronic destructive complication of a latent gingivitis."

In a study of periodontoclasia and its precursory gingivitis the physician and dentist have two important objectives. First, there is the consideration of the probable deleterious effects of paradental suppuration on general health. This doctrine of focal infection is generally recognized and appreciated by both branches of the healing art. Everyone grants that occasionally a diseased tooth or its supporting structures may be the source of joint, nerve, muscle, or eye disease. Nevertheless, any series of cases of arthritis or other chronic disease will yield a disappointingly large percentage of therapeutic failures if sole dependence is placed upon exodontia. The multitude of edentulous young and middle-aged sufferers of chronic disease attest the enthusiasm or despair of those who emphasize the rôle of dental and paradental disorders in systemic disease.

The second objective is that of etiology.

The authors group the conditions, gingivitis and periodontoclasia, because of their identical underlying etiology. The difference represents the time factor

*Received for publication, June 27, 1935.

or duration and the extension of the process from mucous membrane to bone or vice versa. To be sure, certain cases of gingivitis are acute. Some of these are of bacterial origin and are cured by proper local treatment. The majority of the acute and practically all of the subacute and chronic cases, particularly latent involvements of the alveolar gingivae, are not of the infectious type, however. In these nonparasitic forms, the pathologic process develops in such a way as to interfere with the integrity of the peridental structures. The occurrence of subclinical gingivitis is not uncommon and may be overlooked until dental roentgenographs are studied. Frequently, radiographic studies of the alveolar bone have demonstrated extensive resorption in individuals whose mouths were reported to be healthy. Later, carefully taken histories and reexaminations revealed information consistent with Delater's description of the disease.

Etiology.—A study of alveolar gingivitis and its end-result, periodontoclasia, resolves itself into an evaluation of the local etiologic factors and the systemic disturbances which impair the nutrition of the gums and tooth supporting structures. Since all these tissues are constantly traumatized, the process of cell rebuilding must equal that of degeneration. Where nutrition is impaired or curtailed, the catabolic phase is dominant. In periodontoclasia the picture is that of degeneration or failure of regeneration.

In our work at the Tufts College Dental School Clinic, emphasis has been placed on the circulation of the gingivae and deeper structures. In this region there is an enormous capillary bed which is constantly undergoing change in response to physiologic stimuli. The arteries supplying this capillary field are innervated and the arterioles are subject to dilating and constricting influences. Disturbances of the vagosympathetic system therefore improve or embarrass the paradental blood supply. Arteriosclerosis, general or local, lessens the flexibility of gingival and adjacent tissue circulation. This sclerosis may be the sole cause, a complicating factor, or the end-result of the common pathogenesis of periodontoclasia.

Other conditions which are analogous to vascular changes, in that they deplete the tissues, are the blood dyscrasias. Depending upon the severity of the disease, the oxygen and nutritional supply are lessened and there is interference with the waste removal.

Actually, in this series of cases, the authors have found that the circulatory disturbances were the chief etiologic factors.

In the six-year study of 5,446 mouths for Vincent's infection, one of the authors[2] has reached the conclusion that most cases of gingivitis have some etiologic factor which is intimately concerned with systemic dysfunction. He stressed this fact both in acute and chronic types of the condition, and attempted to consider all possible local and general factors. It appeared, however, from the preliminary work presented in that communication that a means of diagnosis and treatment of many of our "failure" and "chronic" cases, both gingivitis and periodontoclasia, might be developed.

The chief difficulty in our approach to the study of these conditions was the necessity of a single diagnostic procedure which would aid in classifying these patients. Eventually it was discovered that the differential leucocyte count

of blood obtained from the gingivae would serve this purpose. The routine examination of gingival blood served to divide the patients into two groups, those in whom the gingival smears were identical with ear or finger tip capillary blood smears; and second, those in whom there was a local oral eosinophilia. Among the first group were found those patients whose cell nutrition was impaired by organic disease. Arteriosclerosis was the most common disease noted. This was a part of the picture of diabetes mellitus in four cases but in the majority it was related to the extensive vascular degenerative process ordinarily manifested by changes in the brain, kidney, and heart. Disturbances of cholesterol balance and endocrinopathies through their effect on the autonomic nervous system were thought to be the etiologic factors in four cases.

Allergic Nature.—In man, the common alterations of tissue function resulting from contact with a protein to which the individual is hypersensitive are: spasm of smooth muscle, spasm of arterioles and arteries, dilatation of capillaries, increased capillary permeability, and local increase of tissue fluids.[3] The allergic response is not universal but may be strictly confined to one organ or even part of an organ. In bronchial asthma, for instance, there is spasm of the bronchiolar muscle; in gastrointestinal food hypersensitiveness there is spasm of the intestinal musculature and, in some forms of angina and myocardial conduction disturbances, there is probably a spasm of the coronary arteriole.[4] The manifestations of allergy may be acute, remittent, and chronic.[5, 6]

Aware of the fact that the allergic diathesis is a congenital biologic alteration of the living cell which changes the capacity of the organism to react to infection and intoxication, it seemed reasonable at least to presume that there might be an allergic factor in the genesis of some oral conditions. Furthermore, the vast majority of proteins which come in contact with the cells of the body enter through the mouth.

The recognized syndromes due to protein hypersensitiveness have multiplied in the past few years. When hay fever and asthma were thought of as the only allergic diseases, it was stated that approximately 10 per cent of the population had an inherited idiosyncrasy of this sort. As other functional disturbances were investigated the morbidity rate of allergic disease rose and in the light of present research one is led to speculate that the constitutional make-up of almost all predisposes to vulnerability of certain tissues to protein intoxication. In addition, the luxuries of civilization, the variety of diet, the extensive cuisine of our modern life, and finally the frequency of acute infections with their consequent bacterial allergy or hyperergy multiply enormously the possibilities of activating a latent tendency to hypersensitiveness.

The study included 86 cases of periodontoclasia, 27 cases of acute, subacute, and chronic gingivitis, and 10 cases of that form of gingivitis commonly found in the latter months of pregnancy.

Investigation of the history of these patients revealed the incidence of some form of allergic disease, actual or presumed, in 46 of the periodontoclasia cases and 16 of the gingivitis cases. The occurrence of asthma, hay fever, migraine, urticaria, serum sickness, or infantile eczema in the patient's or a relative's past life was considered to be actual evidence of an allergic diathesis. A his-

tory of vague gastrointestinal disturbances unexplainable by the ordinary diagnostic procedures was interpreted as a probable sign of protein hypersensitiveness.

History and examination revealed the facts that canerum oris was present in or had been experienced frequently by 34 of the periodontoclasia patients and 21 of the gingivitis cases.

The entire group had friable gingivae, a tendency of the gums to bleed easily when traumatized, and in the gingivitis series local discomfort varied from tenderness to severe pain in some cases.

Each patient was instructed to clean the mouth with castile soap and dry with absorbent cotton. Then the free margin of the gingivae were stimulated with a scaling instrument and, when bleeding was free, blood was transferred directly to and smeared on a glass slide. The preparations were fixed by heat and stained with Wright's methylene blue eosin preparation.

Local gingival eosinophilia was detected in 74 of the 86 cases of periodontoclasia and in each case of the gingivitis group. A study of the oral bacterial flora was inconsequential in the periodontoclasia group. In 6 cases of gingivitis, smears were typical for acute Vincent's infection. In 3 of these patients, the gingivitis preceded the infestation of Vincent organisms by four days.

In addition to the gingival eosinophilia, another finding of consequence in establishing an allergic etiology was the relatively low incidence of polymorphonuclear neutrophiles and the preponderance of the nongranular white corpuscles in the blood smears from these cases. Specimens of blood taken from ear or finger puncture wound were found to be normal and did not reveal the variations of the several types enumerated above. The remaining 13 cases of periodontoclasia had normal leucocyte counts by both technics.

A control group of 35 patients who were afflicted with allergic asthma, hay fever, or urticaria were studied for the incidence of gingival eosinophilia. Each patient had normal gums and peridental structures. In no instance did the differential leucocyte count of the gingival blood vary from that of blood obtained by skin puncture.

A second group of 20 controls, young, healthy nonallergic individuals, failed to produce a single gingival eosinophilia.

The minimal local pathologic signs in the 27 gingival inflammations were swelling and redness of the margin and interdental papillae. This extended over the lingual and buccal surfaces in 20, to the buccal mucosa in 8, to the sublingual mucosa in 6, to the palatal mucosa in 7, and to the anterior pillars of the fauces in 1 case. Ulcers of sizes and severity comparable to the degree of the gingivitis were present somewhere on the inflamed surface in 9 of these cases. The breath was foul in each instance.

Six cases of this 27-patient group were termed chronic gingivitis, for the duration of the condition was over six months in each instance. There was congestion and hypertrophy of the interdental papillae and calculus was present. The latter was the ordinary salivary variety in one case, and the serumal or sanguinary form which is associated with periodontoclasia in a second case.

The entire group of 101 patients were subjected to skin tests. The indentation technic was followed and approximately 120 tests for protein sensitization were performed on each individual. The diagnostic allergens included foods, animal emanations, clothing materials, bacterial proteins, and such incidentals as tobacco and dusts.

Eighty-three of the 101 patients responded in allergic fashion to one or more foreign proteins, the vast majority having several idiosyncrasies. Fifteen of the remaining allergic cases had positive skin reactions to a secondary proteose isolated from the individual's urine. The remaining 3 cases, all of which were affected by acute gingivitis, were studied by means of Rowe's[7] elimination diets.

The proteins to which the patients in these groups reacted allergically covered the entire range of foods, emanations, and incidental proteins. Fifty-six per cent of the allergic group had positive tests to one or more of the proteins of wheat, milk, and egg. Thirty per cent were hypersensitive to tobacco. Four patients who were improved by withdrawal of tobacco were cigaret smokers. In view of this finding, and cognizant of the fact that tobacco sensitiveness is not the result of nicotine contact, ten pipe smokers were examined for gingival eosinophilia. In none did it occur.

Three cigaret smokers who were hypersensitive to tobacco were advised to use holders. Five days later, even though the same number of cigarets were being consumed daily, the gingival blood was found to be normal. On this scanty study, it might be assumed that the cigaret smoker dissolves in his saliva some of the tobacco protein to which he is sensitive.

Treatment.—Gingivitis or periodontoclasia associated with protein hypersensitiveness is treated ideally by the exclusion of the reagen or the offending substance. The management of the acute gingivitis is relatively simple when the nature of the protein is established, but the larger number of periodontoclasia cases had suffered such organic damage that allergic control was helpful but not curative.

In these cases of allergic disease, where the diet could not be controlled or harmful contacts could not be severed, two other procedures were of value. First, the use of mineral oil before meals proved to be a prophylactic measure.[8] Theoretically, oil prevents the absorption of undigested proteins from the gastrointestinal tract and holds them in suspension for further digestion or elimination. This method has worked out well in some cases of wheat sensitiveness where deprivation of wheat would be an economic impossibility or a nutritional hazard.

The second form of palliative treatment employed with success in those cases demonstrating a toxic urinary proteose was the oral administration of the glyceryl esters of ricinoleic acid.

CASE HISTORIES

Since the tabulations (Table I) were made, another series of 26 cases has been completed. Twenty of these were definitely allergic in nature. Because of their number it is impossible to present case reports of all, but it seems to be of value to note the case histories of six fairly typical patients.

C. A., a twenty-one-year-old male, was referred to the clinic for a recurrent severe gingivitis. His first attack occurred four years ago. It consisted of swelling, ulceration and extreme pain in the gingivae. The lesions at that time and in each subsequent attack extended to the buccal surface of the lips, cheeks, pillars, and posterior pharyngeal wall in that order. Because of the discomfort, eating was impossible as the disease progressed. After a period of six to eight weeks, and only when the patient had been positively limited to a milk diet, the condition improved. The patient was seen by the authors two days after the onset of his sixth attack. In blood smears from the gingivae 64 per cent of the white cells were found to be eosinophiles. Skin tests were performed and four-plus twenty-four-hour positive reactions to wheat proteose, globulin, and glutenin were obtained. These three allergens were then applied to a small area of unaffected buccal mucosa and within fifteen minutes a severe reaction was obtained at the site of wheat globulin inoculation.

The patient was instructed to exclude all wheat from his diet. The condition began to subside in two days and was completely cured in five days.

G. D., a twenty-six-year-old female, presented herself at the clinic with a similar history. Her gingivitis, however, had been of eight years' duration. Food protein tests were negative. Bacterial studies had been made and a *Staphylococcus aureus* isolated. With this she had been immunized. No improvement in her oral condition had followed, however. Using bacterial proteins,we obtained a very strong reaction, both on the skin and oral mucosa, to *Streptococcus viridans*.

TABLE I

	NO EOSINOPHILIA	EOSINOPHILIA	ALLERGIC HISTORY	CANCRUM ORIS	POSITIVE SKIN TESTS	URINARY PROTEOSE	ROWE'S DIETS	CURES	IMPROVED
Periodontoclasia	–	74	46	34	62	10	2	16	24
Gingivitis	12 0	27	16	21	21	5	1	22	5

Desensitization was recommended and the patient was referred to her private physician. At the present time, treatment is incomplete and a report on her progress cannot be made.

R. S., a three-year-old male, was referred by his father, a dentist, because of a severe gingivitis of three weeks' duration. The gums became sore, painful, and swollen suddenly. Fever, inability to eat and prostration were constitutional symptoms. Because of the age, local discomfort and uncooperativeness, blood smears were not obtained and skin tests could not be done. Questioning, however, revealed the fact that the child had been presented with a puppy the day of onset. Disposal of the dog was followed by the child's prompt cure. Three months later the child was exposed to another dog. The acute gingivitis recurred.

C. O'B., a thirty-year-old female school-teacher who was temporarily employed in a silk dress factory as an inspector, complained of tenderness and bleeding of the gums, sensitive teeth, and a peculiar taste in the mouth. Roentgenographs of the teeth showed considerable resorption of the alveolar bone. The gums were hypertrophic and bled easily on pressure. There was a gingival eosinophilia of 18 per cent.

Skin tests revealed only one positive reaction, silk protein. The patient then stated that while working as a dress inspector for the past two years she had been in the habit of biting off loose silk threads and had kept them in her mouth for considerable lengths of time.

A. B., a fifty-four-year-old woman, was referred to the clinic for pyorrhea. For several years she had experienced gingival pain on eating very cold or hot foods. The gums bled easily and were sensitive to pressure. Roentgenographs demonstrated considerable alveolar resorption but not sufficient to require a full mouth extraction. There were many gingival pockets from which purulent material and débris could be expressed. There were 17 per cent eosinophiles in the gingival blood smear. She was found to be skin positive to the proteins of tomato, apricot, and yeast.

The patient returned to the clinic in one month in a worse condition than when first seen. Investigation revealed the fact that she had been eating bakers' bread. Two months later, after having been on an absolutely yeast-free diet, the gums were tremendously improved, no purulent matter could be expressed, the interdental tabs were firm and attached, and bleeding on ordinary pressure was absent.

E. B. M., a thirty-eight-year-old mother of five children, was referred for extraction of all remaining teeth.

The first and third left lower molars had been removed five years previously. An extreme degree of pyorrhea existed in the entire right upper and lower molar regions, the process being less pronounced as the midline was approached. Gingival blood studies were interesting in that the degree of eosinophilia corresponded to the extent of pyorrhea. It varied from 28 per cent in right upper and lower second molar region to 12 per cent in the right lateral incisor region to 8 per cent in the gingivae opposite the second left bicuspid. Skin tests revealed a hypersensitiveness to lactalbumin. The interesting feature from the point of view of local trauma and local absorption was the fact that this patient did all her chewing on the right side.

COMMENT

In discussing the probable allergic basis of some forms of gingivitis, one first recalls the frequency of acute lesions in the mouth which follow the ingestion of certain foods. Many patients volunteer the information that canker or soreness of the gums invariably follows the eating of some particular food. Most of these occurrences have been attributed to faulty digestion or inadequate elimination and treated with those conditions in view. Actually, such a form of therapy may be rational, for, if the protein is not quickly altered by the intestinal ferments nor rapidly eliminated, the toxic cleavage product of incomplete protein digestion will be assimilated and cause a reaction. On the other hand, since it is well known that mucous membranes of allergic individuals absorb an offending protein more rapidly than the normal, it may pass through the oral mucosa and exert its effects locally and immediately. The incidence of jagged teeth, overhanging fillings or crowns, malocclusions, and the resultant trauma enhance the absorption of proteins through the abraded or irritated surfaces.

In periodontoclasia, the capillary dilatation, increase of tissue fluid, and spasm of the arteries and arterioles account for the primary alteration of the gingivae and the formation of calculus. With continuance of the arterial spasm the vessels become sclerosed, the blood supply is insufficient, and there is atrophy of the tissues which are dependent upon these vessels for their nutrition. The peridental fibers break down and cannot be replaced because the proper nutrition is lacking. Manipulation of the gums and removal of calculus help by relieving pressure and temporarily improve circulation by the counterirritant effect of the local trauma. Permanent improvement is rare, however, for a vicious cycle has been established.

Ten cases of gingivitis of pregnancy were studied. In six cases, a local gingival eosinophilia was present. All six had several positive skin reactions to proteins and each was allergic to lactalbumin, lactglobulin, or casein. On such slight evidence, we hesitate to draw conclusions. Nevertheless, when one realizes that milk proteins are very common allergens, he questions the theoretical necessity of large amounts of milk in the gravid woman's prescribed diet.

In summarizing these cases, which we believe to be of an allergic nature, it may be said that they have certain diagnostic features in common: eosino-

philia, capillary dilatation, increased permeability, exudation. Other important findings in several cases were: history of food idiosyncrasies or familial predisposition, positive skin reactions, and positive skin tests with urinary proteose.

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BLOOD GROUPING IN THE INFECTIOUS DISEASES*

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IT HAS been maintained for some time that there is a constitutional factor in susceptibility to infectious disease. One avenue of approach to the study of this problem has been a statistical investigation of the incidence of the four blood groups in patients with the various infectious diseases.

A number of such studies have been published. In acute anterior poliomyelitis, Grooten and Kossovitch (1930) noted a marked predominance of Group A over Group O, a complete absence of Group AB, and a slight diminution of Group B, among 78 cases in Alsace. Manicatide, Bratescu, and Rusescu (1929), and Marinesco, Manicatide, and Draganesco (1929), agreed upon a predominance of Group A in Roumanian cases. Foa (1931) reports an appreciable increase in A, and a marked decrease in B, among 22 poliomyelitis patients in Italy. Jungeblut and Smith (1932) report a statistically significant decrease in B and a slight increase in O group, in 125 paralytic cases of poliomyelitis in children over five years of age in New York City. They find a similar decrease in B among 208 polioconvalescent serums. Totalling the findings from the serums and cases, in 551 bloods there are 10.3 per cent B, a decrease from their controls of 3.7 ± 1.7 per cent which is almost of statistical significance. It represents the largest group studied and reported. Shaw, Thelander, and Kilgariff (1932), in 100 cases from California, also report a diminution in B, an

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Received for publication, June 27, 1935.

increase in O, and, strikingly, a complete absence of AB. Hatzky (1933) reports 131 cases in which there is a striking increase in B. With the exception of Hatzky, these various authors agree upon a relative decrease in incidence of individuals belonging to B group in patients with poliomyelitis.

In diphtheria, on the basis of Schick tests, Hirszfeld, Hirszfeld, and Brokman (1924), Nowak (1931), and Farjot (1933) all agree there is no difference in immunity to diphtheria in varying blood groups.

In 36 scarlet fever cases, Mironesco and Stefanov (1926) report a somewhat lowered incidence in Group A and a striking increase in Group B. Nowak (1932), studying 232 cases in Vienna, also finds a slight decrease in Groups A and B. On the other hand, Körwer (1932), in Germany, finds in 363 cases a distribution no different than in his control series, while Kiss and Teveli (1930) find relatively large decreases in both A and B in 172 cases of scarlet fever in Budapest.

In rubella and pertussis, the only report found in the literature is the one of Mironesco and Stefanov (1926), in which are reported 64 cases of rubella, with a slight decrease in Group B, and 14 cases of pertussis, none of which belongs to Group B.

We have collected from the transfusion records of Willard Parker Hospital, a New York City Hospital for contagious diseases, the blood groups of patients typed in the years 1932, 1933, 1934, and 1935. There are 440 individuals represented in this list, 29 of these having had double and, in two instances triple, infections. We are indebted to Dr. K. P. Lorenz, pathologist to the Kingston Avenue Hospital, New York City, for an additional 127 typings. This paper is based upon these 567 patients.

It is obvious that a proper evaluation of the distributions of the blood groups in the several contagious diseases, necessitates a comparison with the "normal" distribution. The considerable variation in incidence of blood groups in different races makes it important to control this factor. This is particularly true in a large metropolitan hospital. Whether or not there has been a stabilization of the racial proportions—and of the distribution of the four blood groups—in a large city such as New York, is not certain. Tiber (1930) has suggested that the lower incidence of Group A in his large series from Bellevue Hospital, as compared with the earlier American reports of Moss (1910), Karsner (1918), and Snyder (1929), is due to the relatively high incidence of South Europeans among the patients at Bellevue Hospital. Similarly low figures for Group A are reported, however, by Culpepper and Ableson (1921) in Detroit, by Seeger and Schaefer (1933) in Milwaukee, and by Jungeblut and Smith (1932) from the Presbyterian Hospital in New York, the correspondence of all of which percentages with those of Tiber is very striking.

For our control group, we have used the distribution obtained from 1,000 individuals, typed as prospective donors for patients at Willard Parker Hospital. They are parents, relatives, and friends of the patients in the hospital (professional donors have been omitted), and therefore presumably reflect the racial distribution of the patients for whom they were typed. The incidence of the various groups differs very slightly from those reported by Tiber, Jungeblut and Smith, Seeger and Schaefer, and Culpepper and Ableson.

In 302 cases of scarlet fever, 45.4 per cent belong to Group O, 30.8 per cent to Group A, 17.9 per cent to Group B, and 6.0 per cent to Group AB. The only differences of any magnitude, as compared with the control group, are seen

TABLE I

NORMAL DISTRIBUTION OF BLOOD GROUPS IN AMERICAN CITIES

		NO.	O	A	B	AB
Culpepper and Ableson	Detroit	5,000	44.5%	36.0%	14.3%	5.2%
Seeger and Schaefer	Milwaukee	489	42.1	36.2	13.9	7.8
Tiber	Bellevue Hospital, N. Y. C.	10,000	45.6	36.4	13.5	4.5
Jungeblut and Smith	Presbyterian Hosp., N. Y. C.	1,000	45.6	35.7	14.0	4.7
Authors'	Willard Parker Hosp., N. Y. C.	1,000	47.8	35.3	12.2	4.7

in Groups A and B. In Group A, there is a diminution of 4.5 per cent ± a standard deviation of 3.06 per cent. The decrease in incidence of Group A is of the same order of magnitude as those reported by Nowak who found a decrease of 6.5 ± 4.0 per cent, and Kiss and Teveli who found a decrease of 9.98 per cent. The figures of Mironesco and Stefanov are too few and too inadequately controlled to be of any significance. The series reported by Körwer show no significant changes in any of the groups from his controls. None of these differences (with the possible exception of Kiss and Teveli's, who do not give sufficient data to treat statistically) reach statistical significance. In Group O, the authors and Körwer find a slight decrease, while Nowak and Kiss and Teveli both find a rather large increase. In Group B, Nowak and Kiss and Teveli find decreases, which is opposite to our increase. This increase of 5.7 ± 2.44 per cent almost attains the rigid statistical significance of probability of 1 in 100 that the difference is not a reflection merely of random sampling (i.e., that there are too few cases to allow the laws of chance distribution to operate), its probability being 0.019. In Group AB, the authors, Nowak, and Körwer all find an insignificant increase, while Kiss and Teveli find a slight decrease.

In the other infectious diseases studied, pertussis, rubella, varicella, and diphtheria, we find in all a striking decrease in those of Group B. The numbers of cases of varicella and diphtheria are too small to merit discussion. In measles, the incidence of Group B is decreased by 4.5 ± 2.56 per cent, and in pertussis by 10.4 ± 1.64 per cent. The first value has a probability of only 8 in 100 that the difference is due to sampling, and for the second, p becomes so exceedingly small that it may be said with complete assurance that the decrease is satistically significant. This agreement between measles and pertussis is suggestive when the similarity of the underlying pathologic process is remembered (McCordock and Muckenfuss; L. W. Smith). If one combines the results obtained in these two pathologically similar diseases, the decrease in Group B is found to be 7.0 ± 1.79 per cent, the p for which value is 1 in 10,000. If one compares the Chi square for the distribution of the blood groups in these diseases with the control distribution, it is found to be 12.05, well above the critical value of 11.34 which indicates a probability of 1 part in 100.

This decrease in Group B in measles and pertussis becomes of even more interest when it is recalled that in poliomyelitis, a statistically significant de-

TABLE II

STATISTICAL ANALYSIS OF THE DISTRIBUTION OF THE BLOOD GROUPS IN THE CONTAGIOUS DISEASES

		O				A				B				AB					
DISEASE	NO.	NO.	% σ	DIFF. FROM CONTROL σ	P*	NO.	% σ	DIFF. FROM CONTROL σ	P*	NO.	% σ	DIFF. FROM CONTROL σ	P*	NO.	% σ	DIFF. FROM CONTROL σ	P*	CHI† SQUARE	P**
Controls	1000	478	47.8 ± 1.58	———		353	35.3 ± 1.51	———		122	12.2 ± 1.03	———		47	4.7 ± 0.67	———			
Scarlet fever	302	137	45.4 ± 2.86	−2.4 ± 3.27	0.47	93	30.8 ± 2.66	−4.5 ± 3.06	0.14	54	17.9 ± 2.21	+ 5.7 ± 2.44	0.019	18	6.0 ± 1.37	+1.3 ± 1.53	0.368	8.0	0.045
Measles	130	71	54.6 ± 4.27	+6.8 ± 4.53	0.13	41	31.6 ± 4.06	−3.7 ± 4.33	0.39	10	7.7 ± 2.34	− 4.5 ± 2.56	0.079	8	6.2 ± 2.12	+1.5 ± 2.22	0.497	4.04	0.25
Pertussis	109	59	54.1 ± 4.77	+6.3 ± 5.03	0.21	38	34.9 ± 4.56	−0.4 ± 4.80	0.94	2	1.8 ± 1.27	−10.4 ± 1.64	$<2\times10^{-9}$	10	9.2 ± 2.77	+4.5 ± 2.86	0.117	14.2	0.0
Varicella	38	16	42.2 ± 8.02	−5.6 ± 8.16	0.49	16	42.2 ± 8.02	+6.9 ± 8.15	0.40	4	10.5 ± 4.98	− 1.7 ± 5.18	0.74	2	5.3 ± 3.64	+0.6 ± 3.70	0.873	0.85	0.90
Diphtheria	21	12	57.2 ± 10.83	+9.4 ± 10.97	0.39	7	33.3 ± 10.28	−2.0 ± 10.30	0.85	1	4.8 ± 4.67	− 7.4 ± 4.78	0.12	1	4.8 ± 4.67	+0.1 ± 4.72	0.984	0.13	0.99

*p, the probability that the difference arises merely due to random sampling, is arrived at by dividing the difference by σ, its standard deviation, and then interpolating this quotient in a table of probabilities (Dunn, 1929).

†X^2, Chi square, a measure of the significance of differences between distributions, is obtained as explained in Dunn's paper, "Statistical Methods in Physiology." Differences between the distributions in the blood groups in the various diseases and the control series are significant when X^2 becomes greater than 11.34.

**p, for the Chi square values, is obtained by interpolating in a suitable nomogram (Dunn, p. 341).

crease in incidence of Group B was also found. Measles and poliomyelitis are both diseases of virus etiology; pertussis is at least suspected of having a virus as an etiologic agent. It might prove of interest, therefore, to ascertain the blood groups of a large number of individuals with other diseases of virus etiology, or suspected of having a virus etiology, e.g., variola, yellow fever, epidemic parotitis, and influenza. Should all of these show a comparatively lower incidence among individuals of Group B, this might serve as a serologic approach to the prophylaxis, and possibly therapy, of these diseases, as has been recently suggested for poliomyelitis by Jungeblut (1933).

SUMMARY

1. There have been 567 individuals with the common infectious diseases typed, and the distribution among the four blood groups compared with a normal distribution.

2. In scarlet fever there is a decrease in incidence of individuals of Group A, and an increase in Group B in pertussis, rubella, and perhaps varicella, to which may be added poliomyelitis as studied previously by one of us. There is a significantly decreased incidence in Group B. Pertussis, furthermore, shows a notable increase among individuals of Group AB.

3. A possible relation of these findings to immunity is suggested.

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A HITHERTO UNDESCRIBED MICROORGANISM OF THE ALCALIGENES GROUP*

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A BACTERIOLOGIC study of a hitherto undescribed microorganism recovered twice from the blood stream of a fatal case is recorded, together with a brief description of the case.

A specimen of blood for bacteriologic examination was received from a woman, aged forty-one, who lived in Westchester County, New York. The symptoms reported were: septic temperature (99 to 104° F.), chills, profuse sweating, extreme prostration, and abdominal distention. The erythrocyte count was said to be 4,080,000, and the leucocyte count 13,500. A differential blood count later showed 91 per cent polymorphonuclear neutrophiles, 8.7 per cent lymphocytes, and 0.3 per cent large mononuclears. A diagnosis of typhoid fever was first suggested, but later "undulant fever or a bacteremia" was considered. The patient's blood serum during the third and fourth week after the onset of illness did not agglutinate *B. typhosus*, *B. abortus* (bovine), or *B. tularense*. No microorganism of the enteric disease group was recovered from the feces. The patient died on the forty-sixth day of illness; an autopsy was not performed. Additional information concerning the case revealed that one week prior to her illness she had returned from a visit on a farm in Virginia where raw milk was used. Only a few days before returning home she had dressed a quail and a rabbit which had been killed by a hunter.

BACTERIOLOGIC STUDY

A small gram-negative aerobic bacillus was isolated in pure culture from the blood specimen which was collected one week before death. Coincidentally, a transplant of a microorganism, recovered from the patient's blood eleven days before death, was received for identification. Preliminary tests indicated that the two cultures, designated as Nos. 3348 and 3349, respectively, were probably homologous and belonged to the *B. alcaligenes* group.

Morphology.—The microorganisms were gram-negative rods that varied from coccoid to bacillary forms; frequently a faintly stained transverse central

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Received for publication, July 2, 1935.

band was observed. They occurred singly or in long or short chains; no flagella, capsules, or polar bodies were demonstrable.

Cultural Characteristics.—On plates of meat infusion agar and horse blood hormone agar the colonies were colorless, glistening, opaque, convex, and friable, with entire edges, and on the blood medium there was no hemolysis. In gelatin tubes after forty-eight hours at 37° C. growth was moderate and flaky. No liquefaction occurred during a three weeks' period of incubation. The growth was moderate and of light creamy color on potato slants soaked in a 0.1 per cent sodium carbonate solution, while on the more acid or glycerol potato medium no growth occurred. In meat infusion broth the cultures showed (after forty-eight hours) slight turbidity with a faint ring and a granular deposit which did not disintegrate on shaking.

Biochemical Reactions.—Litmus milk was unchanged. In milk containing either phenol red or brom cresol purple a slightly alkaline reaction was observed. Indol was not formed. Acetyl-methyl-carbinol was not demonstrated and there was no color change in the methyl-red test. None of the carbohydrates (in peptone water medium) commonly used was fermented, but a strong alkaline reaction was produced in the medium. The microorganisms grew best at 37° C., and indifferently or not at all at 20° C. Exposure to moist heat for five minutes at 60° C. resulted in their death.

SEROLOGIC STUDY

Agglutinating serums were produced in rabbits by repeated intraperitoneal injections of the living microorganisms. As difficulty was encountered in obtaining homogeneous suspensions of the microorganisms, the living cultures were ground before making the suspensions for agglutination purposes. Complete reciprocal agglutination was obtained with cultures 3348 and 3349 and their homologous antiserums in dilutions of 1:1,200. These cultures also reciprocally absorbed the agglutinative properties from antiserums 3348 and 3349 (indicating the identity of the two microorganisms). *B. typhosus, B. paratyphosus* A and B, *B. abortus* (bovine), *B. melitensis, B. paramelitensis, B. whitmori, B. bronchisepticus, Actinobacillus lignierèsi,* and *B. tularense* were not agglutinated in the antiserums 3348 and 3349, nor were cultures 3348 and 3349 agglutinated in the antiserums of the microorganisms just mentioned.

PATHOGENICITY

Rabbits, white rats, and white mice were not found to be susceptible. Guinea pigs injected intraperitoneally with the original cultures (growth from one or two eighteen-hour agar slants, suspended in 1 c.c. of physiologic salt solution) exhibited a variable susceptibility. Some of the guinea pigs became acutely ill and died within twenty-four hours to five days; others, showing only malaise or no symptoms, survived indefinitely. The animals that died of an acute infection showed evidence of acute peritonitis. The microorganisms were cultivated from the peritoneal exudate, the liver, spleen, and mesenteric lymph glands. The passage cultures proved to be infectious for guinea pigs upon intraperitoneal injection.

CLASSIFICATION

Before classifying cultures 3348 and 3349, certain of their cultural and biochemical characteristics were compared with those of the microorganisms which they resembled in many respects, and differences from these same microorganisms (members of the abortus melitensis group and *B. bronchisepticus*) were noted.

There was a striking resemblance between the microorganism and *B. bronchisepticus* in growth on solid media and in morphology, but they differed in many important respects. As Wilson[1] reports, *B. bronchisepticus* produces an intense alkalinity in litmus milk, the alkalinity beginning at the surface and progressing downward until finally the medium is of a blue black color. On potato medium the growth is abundant, moist, and glistening, and of a light tan color after twenty-four hours; in two or three days the color is coffee or Van Dyck brown, the potato itself becoming dark brown. Cultures 3348 and 3349 did not produce a detectable color change in litmus milk after three weeks' growth. They grew on potato slants only if the slants had been soaked in sodium carbonate solution. The growth on this medium was moderate after four or five days and was of a creamy color. Also, *B. bronchisepticus* is a motile bacillus, whereas cultures 3348 and 3349 were nonmotile. However, the failure to demonstrate motility in the two microorganisms is not of great significance as it is a well-known fact that variations of this type may occur in many species of motile bacteria.

While cultures 3348 and 3349 resembled members of the abortus melitensis group in certain of their cultural and biochemical reactions, they were immunologically unrelated to this group and did not produce a chronic disease in the guinea pig. Microorganisms of the abortus melitensis group are serologically related and produce chronic lesions, resembling tuberculosis, in the guinea pig.

The *B. alcaligenes* group is generally considered to be composed of gram-negative bacilli that do not ferment carbohydrates and do not form acetyl-methyl-carbinol. This unidentified strain fulfilled these criteria. For convenience of reference to the literature, therefore, it seemed advisable to place the microorganism in this group. However, it should be pointed out that the strain has very few characteristics in common with the type species, *B. fecalis alcaligenes*, which is a motile bacillus with peritrichous flagella, produces an intense alkalinization of litmus milk, grows well on ordinary media, and is not sensitive to environmental changes. Cultures 3348 and 3349 are nonmotile, do not possess flagella, produce only a slight alkalinization of milk, are fastidious in their media requirements, and are highly sensitive to environmental changes.

SUMMARY AND CONCLUSIONS

The homologous gram-negative bacilli cultivated from two blood specimens obtained from a fatal case of generalized infection were compared with *B. bronchisepticus* and the members of the abortus melitensis group. The significant differences and similarities between this and the heterologous microorganisms were pointed out. Distinctive morphologic, cultural, and biochemical characteristics indicate that it is a representative of a new and hitherto un-

described species. Because of certain characteristics, the microorganism has been placed within the *B. alcaligenes* group, although it differs in many respects from the type species, *B. fecalis alcaligenes.*

It is entirely possible that infections due to this pathogen have been overlooked because of the similarity of the clinical symptoms to those of other well-known diseases which are characterized by a bacteremia.

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NORMAL MAGNESIUM METABOLISM AND ITS SIGNIFICANT DISTURBANCES*

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ALTHOUGH for many years satisfactory methods have been available for the estimation of magnesium in body fluids, such determinations have received little attention from clinical chemists. Our attempt in this paper is to review briefly some of the more important physiologic relationships of magnesium, note certain variations in abnormal states, as well as present a series of magnesium determinations on healthy and diseased individuals. We wish to emphasize in particular the retention of magnesium which occurs in some cases of renal disease, a fact denied by some writers on the subject, and its significance in diagnosis, prognosis, and treatment.

Since practically all foods in the human dietary contain an adequate amount of magnesium to meet the body's requirements (Joachimoglu and Panopoulos[1]), the problem of specific deficiency in magnesium does not arise in human nutrition. The effect of magnesium deprivation in animals, leading to a fall in serum magnesium and the typical magnesium tetany, has been observed and described by the group in E. V. McCollum's laboratory.[2, 3, 4, 5]

Numerous attempts have been made to associate dietary deficiency in magnesium with increased incidence of malignant neoplasms. This subject is thoroughly reviewed by M. J. Shear[6] who finds the evidence contradictory and insufficient.

In regard to the effects of excessive magnesium intake, the only study with human subjects which has come to our attention is that of Carswell and Winter;[7] their experiments lasted twenty and twenty-four days, and consisted of the addition of 8 gm. of magnesium lactate to a uniform diet adequate in phosphorus. They were able to show that about half of the ingested magnesium was absorbed. Calcium balance was positive in both cases, arguing against the

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Received for publication, July 4, 1935.

concept that excessive magnesium intake conditions a storage of magnesium at the expense of calcium. Magnesium was stored in both experiments, but not to the detriment of the calcium reserve.

The difficulty in studying the effect of oral administration of excess magnesium lies in the fact that the intestine absorbs it only to a partial and variable extent (Elmslie and Steenbock[8]). McCarrison[9] and Watchorn[10] have succeeded in producing renal calculi in experimental rats, by diets in which magnesium was excessive. Georg Meyer zu Hörste[11] has produced a rachitis by deficiency of phosphorus in rabbits and rats, similar to that obtained by excess calcium, by feeding excess magnesium carbonate.

The results of parenteral administration of magnesium fall into the widely-studied pharmacological field of the narcotic and anesthetic effect of magnesium, which is beyond the scope of this article. Some concepts of physiologic significance can, however, be drawn from such experiments, such as the possibility of a central depression of respiration by excess blood magnesium (Trabucchi and Gazzari[12]), and the observation of increase in serum magnesium (with decrease in calcium) in narcoses induced by other agents than magnesium, e.g., ether and chloroform (Pineussen and Dimitrijevic[13]).

We know that magnesium is essential in the enzymic fermentation of carbohydrate, whether by yeast (v. Euler, Nilsson and Auhagen[14]), or by muscle (Lohmann[15]). The phosphatases also require magnesium (Jenner and Kay,[16] Erdtmann[17]). Marked increases in serum magnesium can be produced in experimental animals by electrically induced strenuous muscle activity (Waeker[18]).

Parathyroid hormone has been shown to condition a rise in blood magnesium (Scholtz,[19] Greenberg and Mackey[20]). Magnesium is related, in a manner not yet clearly defined, with the function of the generative organs, especially in the female, and possibly of the anterior lobe of the hypophysis. Watchorn[21] in 1926 observed variations in human serum magnesium in connection with menstrual irregularities. In certain of the animals of her later series[10] where the main observation dealt with the formation of renal calculi, congestive turgor of the uterus and ovaries was noted, with excessive formation of *corpora hemorrhagica*. The changes were not associated with estrus, as determined by vaginal smear, and were in excess of those associated with estrus in the normal rat. There was no correlation with the formation of renal calculi.

In experimental animals (dogs and rabbits) there is a marked increase in serum magnesium following injection of prolan, at the expense of the magnesium of certain organs (brain, kidney, testis, lung). This could not be duplicated in human subjects (Cannavo,[22] Cannavo and Indovina[23]).

The increase in magnesium content of teeth during the progress of caries has been repeatedly pointed out (Howe,[24] Kaushansky[25]).

The increase in serum magnesium noted in oxalic acid poisoning (Jacoby and Friedel[26]) is probably sufficiently explained as compensatory for the decrease in calcium.

The elimination of magnesium is well known to be both through the urine and the feces. The bile, as well as the intestinal mucosa, contributes to the latter source of excretion (Delbet and Breteau[27]). The magnesium of the bile

is claimed to add to its solvent properties for cholesterol (Delbet and Beauvy[28]) and may have a part in the emptying mechanism of the gallbladder (Erbsen and Damm[29]).

A portion of the serum magnesium has been shown to be in a combined and nondialyzable form (Bernhard and Beaver[30]). Watchorn and McCance[31] set the dialyzable fraction at 75 per cent, with no significant variations with changes in quantity.

The magnesium content of the cerebrospinal fluid being consistently higher than that of serum (Cohen[32]) has been a recurrent obstacle in the attempts at explaining the formation of cerebrospinal fluid on the basis of ultrafiltration (Stary, Kral and Winternitz;[33] Massaut;[34] McCance and Watchorn[35]).

Retention of magnesium in renal insufficiency, with increased blood serum levels, has been denied (Fishberg,[36] Denis and Hobson[37]) as well as affirmed (Becher[38]). This will be discussed further after presentation of our own data.

Methods.—Our methods were modifications of those of Briggs.[39] The differences, in the blood method, consisted of precipitation directly from serum without use of a trichloracetic acid filtrate, and of the use of the Fiske and Subbarow[40] reagents for the final colorimetric estimation. Calcium was removed as in the Kramer and Tisdall[41] method. The procedure for blood serum is as follows: To 2 c.c. serum were added 2 c.c. water and 1 c.c. 4 per cent ammonium oxalate solution. After mixing, the tubes were allowed to stand one-half hour, then centrifuged. The entire supernatant liquid was poured off and used for the magnesium determination. (The residue could be used, if desired, for calcium determination.) To the supernatant liquid in another centrifuge tube were added 1 c.c. of 2 per cent ammonium phosphate solution and 1 c.c. concentrated ammonia water. After mixing and standing for four hours, the precipitate of magnesium ammonium phosphate was separated by the centrifuge, and washed once with ammoniacal alcohol (according to Briggs, *loc. cit.:* 200 c.c. of 95 per cent alcohol and 50 c.c. of concentrated ammonia water diluted to one liter). After washing, the precipitate was dissolved in 5 c.c. of 2.5 N sulphuric acid, transferred to a 25 c.c. volumetric flask, and treated with 2.5 c.c. Molybdate III (according to Fiske and Subbarow, *loc. cit.:* 2.5 per cent of ammonium molybdate in water) and 1 c.c. of the Fiske and Subbarow reducing agent. After five minutes for color development, this was made up to volume and read against a standard phosphate solution (10 c.c. solution containing 0.051 mg. P as KH_2PO_4, equivalent to 0.04 mg., Mg as $MgNH_4PO_4$) treated in a similar volumetric flask with the same amounts of 2.5 N sulphuric acid, molybdate solution, and reducing agent.

In the urine, following Briggs's suggestion, calcium and magnesium were precipitated simultaneously. Two cubic centimeters of urine in a 15 c.c. graduated centrifuge tube was made acid to Congo paper by the addition of one or two drops of hydrochloric acid. Water was added to 5 c.c. volume, followed in order by 1 c.c. of 4 per cent ammonium oxalate, 1 c.c. of 2 per cent ammonium phosphate, and 2 c.c. of concentrated ammonia water. After four hours' standing the precipitate was separated by the centrifuge, washed once with ammoniacal alcohol, and dissolved in 3 c.c. of 2.5 N sulphuric acid. (At this point, if desired, the calcium could be titrated with N/100 permanganate.) To

the dissolved precipitate, still in the centrifuge tube, were added water to 10 c.c., 1.5 c.c. Molybdate III, and 0.6 c.c. of the reducing agent. After five minutes for color development, the volume was made up to 15 c.c. and the color compared with that of a simultaneously prepared standard made in a 100 c.c. volumetric flask by adding, in order, 5 c.c. standard phosphate solution (containing 0.4 mg. P equivalent to 0.314 mg. Mg), 50 c.c. water, 20 c.c. 2.5 N sulphuric acid, 10 c.c. Molybdate III, 4 c.c. reducing agent, and water to 100 c.c. volume.

These older methods were used from simple personal preference to the newer 8-hydroxyquinoline methods. The results have been shown to be identical (Greenberg and others[42]). Numerous colorimetric methods based upon the use of dyestuffs have been proposed (Becka,[43] Lang,[44] Thrun[45]). The last two were tried, but the results were not as consistent as with the magnesium ammonium phosphate precipitation.

Normal Blood Values.—Eighty-seven determinations were made, using blood serum obtained from students in the medical school. The values obtained varied between 1.6 and 3.0 mg. per 100 c.c. serum, with a mean value of 2.2 mg. The values are somewhat lower than those obtained by Greenberg and others[42] in a similar series, and more in accordance with those observed by Becher[38] and by Bomskov[46] and those stated by Shohl.[47] Greenberg's mean value is 2.74, his range 2.0 to 3.6; Becher's range is 1.8 to 2.3, that of Bomskov 1.7 to 2.6. Shohl sets the limits between one and three mg. per 100 c.c.

This group may be supplemented by the somewhat less authentic figures derived from analyses made on 91 miscellaneous medical and surgical patients, with no apparent disturbances of mineral metabolism and elimination. Of this group the mean value was 2.3 mg. per 100 c.c.; the range was from 1.5 to 2.9 mg. Wacker and Fahrig[48] in a similar series obtained a mean value of 2.28 mg. with a range of 2.03 to 2.97.

Blood samples in both groups were uniformly taken in the morning before breakfast.

Normal Urine Values.—In the group of medical students an attempt was made to correlate urine output over a definite period with the blood levels. This was previously done by one of us for phosphorus (Walker[49]). For magnesium no correlation could be shown. The values for the one-hour excretion varied from 0.3 to 10.9 mg., with an average of 2.6 mg. per hour.

The twenty-four-hour output of magnesium was also observed in the same group; the maximum twenty-four-hour excretion was 307 mg., the minimum 32.5 mg., the mean value 103 mg.

In the group of hospital cases, supposedly normal from the excretory viewpoint, the values noted in the twenty-four-hour collections were definitely lower. The maximum was 243 mg., the minimum 5 mg., and the mean 86.

Abnormal Values.—Certain cases were observed in the hospital at the time of this study with definitely increased levels of serum magnesium.

Mr. A. C., aged forty-six, was admitted Feb. 11, 1934, in a preuremic condition, with a blood pressure of 210/127. He had a convulsion about two hours after admission, lasting three or four minutes, followed by coma. His history was of albuminuria and hypertension for the previous eight years, severe bilateral temporal headache for five weeks and frequent vomiting for the preceding twelve hours. At the time of admission he was disoriented and

restless. Physical examination revealed fresh and old hemorrhages in both fundi oculi, heavily furred swollen tongue, foul breath, heart size and sounds normal. He was treated by phlebotomy, lumbar puncture, and magnesium sulphate by mouth and intravenously. His condition improved two days after admission, and he was discharged much improved on March 17, 1934. Repeated urine examinations consistently demonstrated a tendency to fixation of specific gravity between 1.009 and 1.015. Albumin was present, and the sediment always contained 3 to 10 leucocytes and 2 to 12 blood discs per high power field. Hyaline, granular, cellular, and waxy casts were plentiful in all specimens. Blood examination on admission revealed nonprotein nitrogen 80 mg., uric acid 9.5 mg., creatinine 2.3 mg., and sugar 129 mg. per 100 c.c. The diagnosis was chronic glomerulonephritis with hypertension and uremia. Two determinations of serum magnesium were made in this case; at the time of admission it was 3.92 mg., and three weeks after admission 2.1 mg. This patient had a recurrence of uremia in January, 1935, and died.

Miss S. J., aged fifty-six, was admitted Nov. 26, 1933, for diagnostic study with history of hypertension of fifteen years' standing and of migrainous attacks accompanied by partial loss of vision and fortification scotomata. Examination demonstrated an irregular heart with an aortic systolic murmur and a blood pressure of 222/104. Urine examinations were negative except for a very small trace of albumin and a few finely granular casts. Blood counts were within normal limits. Further cardiac examination failed to confirm the arrhythmia but demonstrated a myocardial impairment suggestive of coronary disease. She was not treated in the hospital. Serum magnesium on admission was 3.10 mg. and a week later 2.84 mg.

Mr. A. L., aged thirty-four, was admitted Sept. 9, 1933, with a history of hypertension and nephritis of two and one-half years' duration, with increasingly frequent attacks of headache, nausea, and vomiting. Admission examination revealed a blood pressure of 204/126 and an apical systolic cardiac murmur. There was evidence of cardiac enlargement downward, confirmed by x-ray. He remained in the hospital at this time until Dec. 3, 1933, during which period his symptoms were not brought completely under control. Urine examinations demonstrated a fixation of specific gravity between 1.012 and 1.014, small traces of albumin, rare leucocytes, frequent showers of blood discs and occasional hyaline and coarsely granular casts. Blood morphology was normal. Chemical examination of the blood revealed nonprotein nitrogen 57 mg., uric acid 9.1 mg., creatinine 2.5 mg., and glucose 91 mg. per 100 c.c.

He was readmitted on March 4, 1934, following a minor accident which brought on a recurrence of the severe headache, nausea, and vomiting. Following another long hospital stay, he was discharged on May 26, symptomatically improved, with a diagnosis of malignant hypertension, hypertensive heart disease, and chronic nephritis. The prognosis was considered very poor. Laboratory findings were comparable to those of the first admission. Therapeutic lumbar puncture was repeated several times and appeared to be the most effective form of treatment. This patient showed a consistently elevated blood magnesium level as shown by the following results: October 30, 2.55 mg.; November 6, 2.07 mg.; November 13, 2.50 mg.; November 20, 2.58 mg.; November 27, 3.26 mg.; December 4, 2.34 mg.; January 22, 3.28 mg.; March 5, 4.05 mg.

Mr. F. R., aged twenty-six, was admitted Oct. 28, 1933, with a history of hypertension, dyspnea, palpitation, and precordial pain for a period of nine months. There had been a suspicion of lead poisoning, not confirmed by laboratory examinations. Examination demonstrated a yellow pallor, bleeding gums, faint systolic apical and aortic murmurs, and a fine tremor of the fingers. During his hospital stay there was a progressive change for the worse in his blood chemistry and other renal function tests. He was discharged against advice December 9, and died shortly after his return home. Blood pressure at time of discharge was not significantly changed. Urine examinations demonstrated a definite fixation of sp. gr. at 1.010, traces of albumin and occasional casts. He had a marked anemia, 2,000,000 erythrocytes per c.mm. on admission, 1,700,000 on discharge, with color indices varying between 0.8 and 1.0. Blood chemical examination showed nonprotein nitrogen increasing from 204 to 263 mg., uric acid from 9.1 to 13.2 mg., and creatinine from 8.8 to 13.6 mg. His blood sugar was within normal limits. Repeated phenolsulphonephthalein tests gave no

return. The progress of the disease is well illustrated by the increasing values of blood serum magnesium (in mg. per 100 c.c.): October 30, 2.53; November 6, 2.41; November 13, 2.76; November 20, 3.85; November 27, 4.00; December 4, 4.20.

Mr. R. I., aged fifty-two, was admitted Oct. 14, 1933, for study of a hypertensive condition. He also complained of dyspnea, cough with bloody expectoration, edema, and right-sided paresis. Physical findings included glossitis, anterior and posterior cervical adenopathy, râles and dullness in the bases of both lungs, enlarged and tender liver and pitting edema of both legs. The blood pressure was 192/112. A diagnosis of chronic lymphatic leucemia was made within a short time following admission on the basis of the blood examination. The urine was found to contain an enormous number of red blood discs. Chemical examination of the blood gave normal figures for the usual constituents. The phenolsulphonephthalein excretion was 38 per cent in two hours. X-ray gave evidence of considerable effusion in the right pleural cavity. There was no fixation of specific gravity of the urine. Neurologic examination suggested a probable earlier cerebral hemorrhage. He was discharged November 4, unimproved. His serum magnesium was 3.30 mg. per 100 c.c. on admission, later normal values of 2.57 and of 2.66 were obtained. He died soon after his discharge from the hospital.

Mrs. E. T., aged twenty-three, was admitted Feb. 14, 1934, for treatment of pernicious vomiting of pregnancy. Her last period was Jan. 7, 1934, and the vomiting had been of two days' duration and of such severity that she had been unable to retain food or fluids. She was suffering from constant thirst, nausea, severe occipital headaches, and sense of pressure in the lower abdomen. During the last year she had had two pregnancies, both of which had been terminated by curettage on account of the excessive vomiting. Physical examination was noninformative except for signs of dehydration and generalized abdominal tenderness and rigidity. Pregnancy was confirmed by the Aschheim-Zondek test. The vomiting was controlled by use of intravenous glucose together with sedation by phenobarbital. She remained in the hospital until March 7 with only one further episode of severe vomiting, which was eventually controlled by gastric lavage. Urine examination revealed leucocytes in varying and sometimes large numbers. Blood morphology was normal as well as routine blood chemical examination. A single blood magnesium determination done on the day of admission was 3.25 mg. per 100 c.c.

Wacker and Fahrig[48] in a series of patients with hypertension demonstrated an increase in serum magnesium from their normal mean value of 2.28 to 2.41 mg. This slight difference hardly appears significant, but it is of interest to note that out of our six cases showing increased serum magnesium five had hypertension, the other being a toxemia of pregnancy. Weil, Guillaumin and Weismann-Netter[50] also have observed increased value of serum magnesium in a few cases of essential hypertension. Their figures deviate so far, however, from the standards used by other authors that comparison is not possible.

In our hospital series there were fifteen other cases of hypertension, none showing any signs of severe kidney damage. Of this group, the maximal magnesium value in blood serum was 2.72, the minimal 1.86, and the mean 2.36 mg. per 100 c.c. This is definitely and probably significantly higher than the means of the normal and of the hospital series (2.2 and 2.3 mg. respectively).

SUMMARY

1. The recent literature on the pathologic chemistry of magnesium has been briefly reviewed.

2. The range of serum magnesium in fasting normal active young adults (87 subjects) is found to be 1.6 to 3.0 mg. per 100 c.c., with a mean value of 2.2 mg.

3. The range in a group of 91 miscellaneous medical and surgical hospital cases with no apparent disturbance of mineral metabolism was 1.5 to 2.9 mg. with a mean value of 2.3 mg.

4. The twenty-four-hour urinary output of magnesium in the student group was 32.5 to 307 mg., with a mean value of 103 mg. For the hospital group the maximum was 243 mg., the minimum 5 mg., and the mean 86 mg.

5. In five cases of hypertension associated with a greater or lesser degree of renal damage, abnormally high serum magnesium values were found, the highest value being 4.20 mg. in a case of terminal nephritis.

6. In a group of cases of hypertension without severe renal damage the maximum was 2.72, the minimum 1.86, and the mean 2.36 mg.

7. It appears that, contrary to certain statements in the literature, serum magnesium may be elevated in moderate or severe renal insufficiency, especially if associated with hypertension.

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ANTIPNEUMOCOCCUS SERUM CONTAINING HETEROPHILE ANTIBODY*

A Laboratory and Clinical Report

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PNEUMONIA in man is common and carries a high mortality. The need of improvement in the treatment of this disease is apparent, and is exemplified by the various studies on the use of antiserums, drugs, pneumothorax, and other agents.

About 90 per cent of all cases of this disease are caused by the pneumococcus, the remaining 10 per cent being due to other organisms.[1] The early work of Avery, Chickering, Cole, and Dochez[2] on acute lobar pneumonia was preceded and followed by many reports on the value and use of various antipneumococcus serums. Recognition of a great multiplicity of types of pneumococcus[3] has enhanced the difficulties of the general preparation and use of pneumococcus antiserum. Considered from the type specific aspect, pneumonia, as caused by the pneumococcus, comprises a group of separate diseases, in contrast to such a disease as diphtheria.

The accumulation of reports on the use of antiserums in pneumonia has given statistical evidence of the clinical usefulness of antiserums of Type I and, to a lesser extent, of Type II. Success in the treatment of pneumonia with antiserums is consequently dependent upon early diagnosis, immediate typing of the infecting organism, and prompt administration of the antiserum of the type indicated, with the further practical limitation that the latter must be either Type I or II.

That this procedure is not entirely satisfactory, and that the delay caused by typing the organisms often proves fatal, is evidenced by the fact that Cecil and Sutliff,[4] and later Park,[5] have advocated the immediate use of a polyvalent antiserum to be followed later by type specific serums.

Recently our knowledge of the antigenic structure of the pneumococcus has been increased by the discovery of Bailey and Shorb,[6] that this organism contains heterophile or Forssman antigen.[7] That the heterophile antibody plays a significant rôle in the defensive mechanisms of the body to pneumonia is suggested by the work of Bailey and Shorb,[6] and by the report of Powell, Jamieson, Bailey and Hyde[8] on the treatment of experimental pneumococcus infection in rabbits with various heterophile antibody antiserums. These authors noted that considerable active and passive antipneumococcal immunity is exhibited by heterophile antibody, and subsequently Bailey and Shorb[9] have reported an increase in the phagocytosis of pneumococci in the presence of combined type specific and heterophile antibodies.

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Received for publication, July 11, 1935.

Since pneumococcus heterophile antigen is species-broad, instead of type specific in distribution, it is evident that such an antiserum, if valuable at all, would be equally useful for all types of pneumococcic pneumonia and would not necessitate typing of the infecting organism. In the past, pneumococcus antiserum that has been used has been mainly of horse origin and hence has contained no heterophile antibody. Therefore, it seemed desirable, in order to throw light on the clinical value of heterophile antiserums in pneumococcus pneumonia, to obtain heterophile antibody in quantity, add some of this to the conventional mouse protective horse antipneumococcus serum, and treat available cases of lobar pneumonia with (a) plain heterophile antiserum, and (b) with a mixed antibody serum.

PREPARATION OF HETEROPHILE ANTISERUM AND ANTIPNEUMOCOCCUS SERUM CONTAINING HETEROPHILE ANTIBODY*

Theoretically, heterophile antigen from any source should be moderately satisfactory for inciting the formation of heterophile antibody for use in antipneumococcus serum. That heterophile antigen of pneumococcus origin should be preferable is indicated by the work of Landsteiner and Levine,[10] and the reports of Bailey[9] and his associates on absorption reactions of various heterophile antigens and antibodies.

Various animals of the "rabbit type" should suffice for the preparation of ample heterophile antibody. From a practical point of view one is limited to the cow and rabbit as animals most suitable for this purpose, since other domesticated animals such as the horse, sheep, and goat are unsuitable, in that their tissues contain normally a large amount of heterophile antigen, and therefore they do not produce heterophile antibody (*i.e.*, these are of the "guinea pig type").

The first attempts to produce suitable heterophile antibody in large quantity were made by using both calves and rabbits in parallel experiments. After six months of immunization, the experiments with the calves were discontinued because of the uniformly low heterophile titers obtained.

Rabbits proved satisfactory for producing antipneumococcus heterophile antibodies. The limitation in rabbit immunization is the small yield of serum per rabbit and the tedious process of immunization of many animals.

However, we have immunized large groups of rabbits, using from twenty-five to one hundred animals at a time. The animals were inoculated with a suspension of heat-killed pneumococci of about 50 per cent of a mixture of equal parts of Type I, II, III, and Cooper's Type IV, plus approximately 50 per cent of a rough strain known as D.R.I., which is very high in heterophile antigen.[6]

A course of immunization of three to four weeks led to the production of heterophile antibody titers varying from 2,500 units to 25,000 or more units per cubic centimeter, and the production of practically no mouse protective anti-

*We are indebted to H. M. Powell of the Lilly Research Laboratory, Indianapolis, for the description of the preparation and for all the antiserums used in the work reported in this paper.

body for any of the types. The mouse tests of course evaluate the type specific Felton antibody, but do not evaluate the species-broad heterophile antibody.

Prior to combining it with horse antipneumococcic serum, we have concentrated our rabbit heterophile antipneumococcic serum by salting out on half saturation with ammonium sulphate. The whole globulin precipitate is filtered off from the albumin solution, the latter discarded, and the precipitate dialyzed until it is salt-free. The resultant heavy precipitate of euglobulin is removed by centrifugation, the solution is made isotonic to normal serum and filtered through a Berkefeld candle. Merthiolate is finally added as a preservative, in a concentration of 1:10,000. Final sterility tests, safety tests, and heterophile antibody potency tests are then conducted in the regular way.

In the preparation of the usual mouse protective antibody, horses have been used. We have utilized for injection Types I, II, III, and Cooper's Type IV pneumococci, of the regular high degree of virulence for mice as maintained by weekly mouse passage. The culture emulsions have been heat-killed, and the horse bleedings on attaining sufficient potency have been concentrated and prepared in the usual way for therapeutic use. Mouse protective tests have showed an increasing potency against Types I and II of from four hundred to five hundred units up to one thousand or twelve hundred units, with a gradual improvement in the serum during the period of its use. Although this serum shows a varying mouse protective potency against certain laboratory strains of Type III pneumococcus, this is discounted by the fact that it shows practically no unitage against our heavily encapsulated Type III laboratory strain known as Park III. So-called Type III antiserums prepared elsewhere have given these same effects in our hands, and little can be expected directly of any weak Type III antibody present, especially in view of previous unsatisfactory clinical experience with Type III mouse protective serum.[11] Such "weak" Type III antibody is greatly increased in opsonic action, however, by the addition of heterophile antibody,[12] and such a combination of antibodies is theoretically deserving of a clinical trial. Little can be said at this time about the single Type IV qualities of this serum.

A combined serum was obtained by mixing together heterophile antipneumococcus serum and mouse protective antipneumococcus serum. Generally one-fourth to one-tenth of the mixed serum has comprised heterophile antibody while three-fourths to nine-tenths of the volume has comprised mouse protective antibody. The mixing of the two antiserums has been done in a fractional way and the resultant precipitate of heterophile antigen antibody complex (the heterophile antigen coming from the horse serum) has been removed by filtration, before addition of more heterophile antibody.

The various lots or batches of combined heterophile and mouse protective antipneumococcus serum prepared for clinical use as reported below have varied from original potencies of 500 heterophile units and 400 mouse protective Type I and Type II units per cubic centimeter, up to 10,000 heterophile units and 1,000 mouse protective Type I and Type II units per cubic centimeter. It was therefore an expectation that clinical results would be better as the work progressed, all other factors being approximately equal.

CLINICAL USE AND RESULTS

The patients treated with the above described antiserums were cases at the Cincinnati General Hospital. Their ages varied from sixteen years to seventy. During the period of time that we were treating these patients, *every case of pneumococcic pneumonia was treated and is included in our figures* with the exception of a very few which were not treated solely because of an occasional shortage of antiserums. We did not select our cases; those of old as well as of recent onset were treated; and we did not exclude from our figures those patients who died within twenty-four hours after their admission to the hospital. It

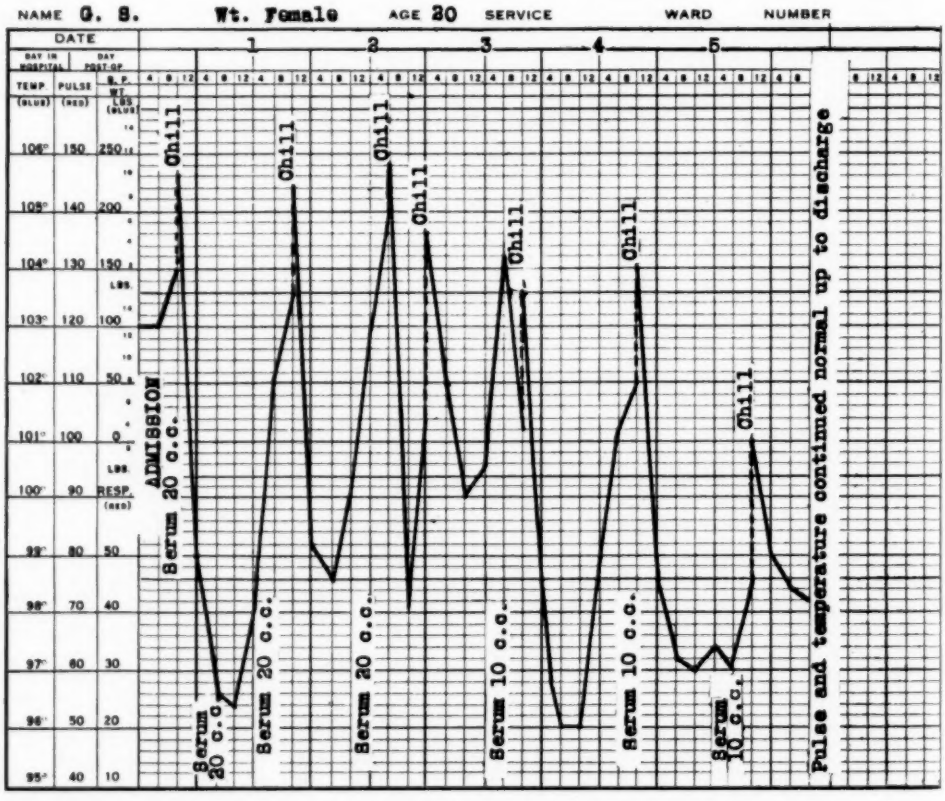

Chart 1.

must be admitted that human pneumonia is such a variable disease from case to case and time to time that only approximate results could be obtained in a statistical way, but spectacularly good results with the heterophile antiserum treatment of case after case would be strong presumptive indication of effectiveness. We are reporting these cases, not for the purpose of giving definite conclusions, but merely to give our own results, on account of the importance of this subject.

When the patient was admitted to the hospital, if a diagnosis of lobar pneumonia was made, an x-ray picture of the chest was obtained, a sample of the sputum was collected for culture and typing by the mouse method, and the

antiserum was given immediately. The patients were tested for sensitiveness to the serum before any of it was given. Only one case was found sensitive, and this individual was not treated, or included in our data. All of the serum was given intravenously. The amount of serum varied, the initial dose being from 10 to 60 c.c. of the concentrate. The serum was given once or twice daily. The total amount of serum given to one patient varied from 40 to as high as 580 c.c. The typical response was a rise in fever with an occasional chill, followed by a rapid fall in temperature to normal or even subnormal. Two such temperature curves are shown in Charts 1 and 2. Not all of the cases responded in this way.

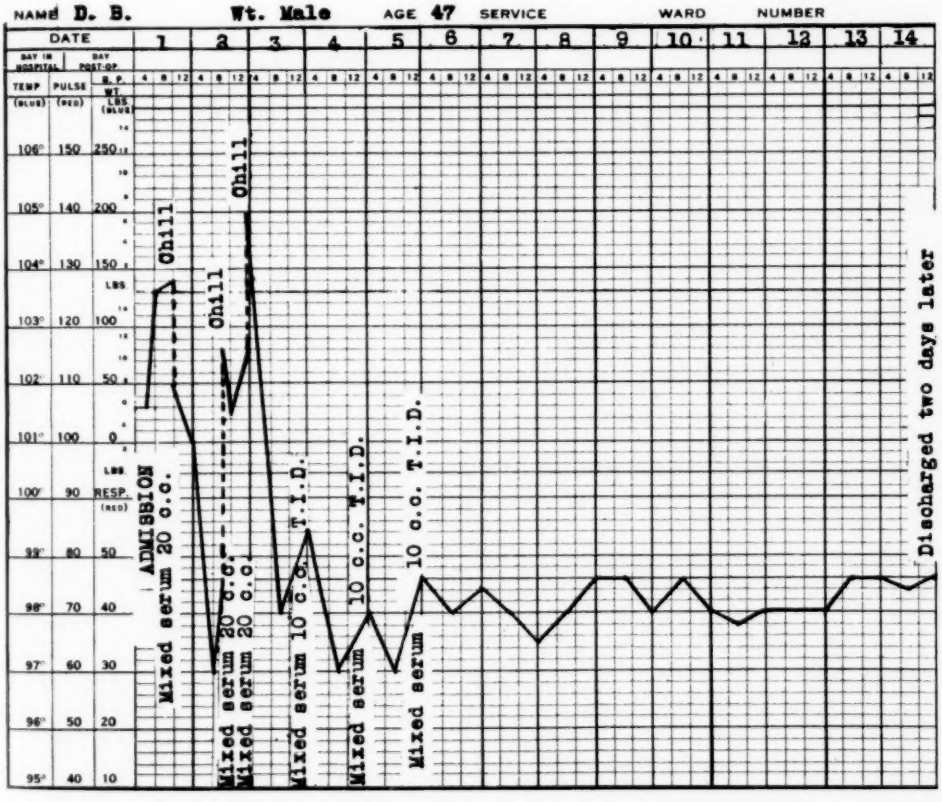

Chart 2.

In order to see if normal rabbit serum concentrate would produce a similar reaction it was given to three pneumonia patients. There was no noticeable reaction. They did not have a temperature rise or chill. One of these patients

TABLE I

SHOWING RESULTS OF PNEUMONIA CASES TREATED WITH HETEROPHILE ANTISERUMS (JANUARY TO APRIL, 1933)

	NO.	DIED	RECOVERED	MORTALITY PERCENTAGE
Total	34	16	18	47.0
White	16	6	10	37.5
Black	18	10	8	55.5

was given heterophile antiserum after two injections of normal serum concentrate had failed to produce any reaction or chill. After the heterophile antiserum he had an increase in fever and a chill. This is shown in Charts 3 and 4.

From Jan. 10, 1933 to April 1, 1933 thirty-four patients were treated. The results are shown in Table I.

From Table I, it will be noted that the mortality among the colored patients was higher than for the white patients. Among the sixteen patients who died,

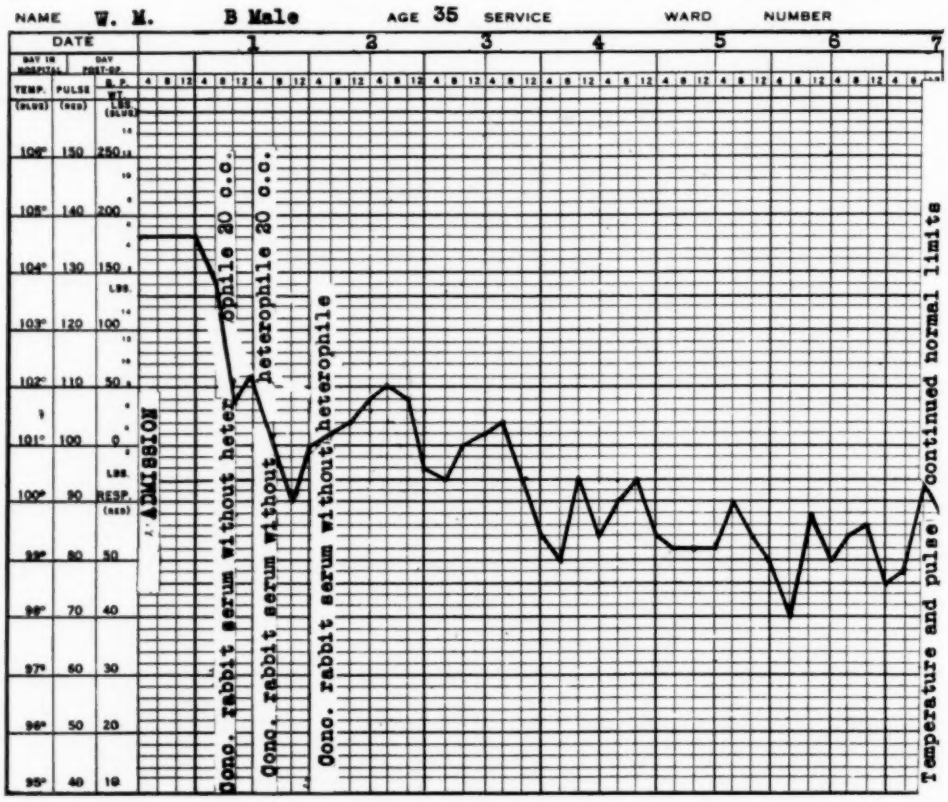

Chart 3.

four did not receive their first antiserum until more than ninety hours after the onset of the disease, one died eighteen hours after admission, and one was a chronic alcoholic.

TABLE II

SHOWING CASES TREATED WITH PLAIN AND COMBINED HETEROPHILE SERUMS (JANUARY TO APRIL, 1933)

		NO.	DIED	RECOVERED	PERCENTAGE MORTALITY
Treated with plain serum	Total	16	8	8	50.0
	White	8	3	5	37.5
	Black	8	5	3	62.5
Treated with combined serum	Total	18	8	10	44.4
	White	8	3	5	37.5
	Black	10	5	5	50.0

Sixteen of these thirty-four patients were treated with plain heterophile serum, while eighteen were treated with mixed heterophile and type specific serum. The separate results are shown in Table II.

The results in Table II suggest that the combined serums might be slightly more effective.

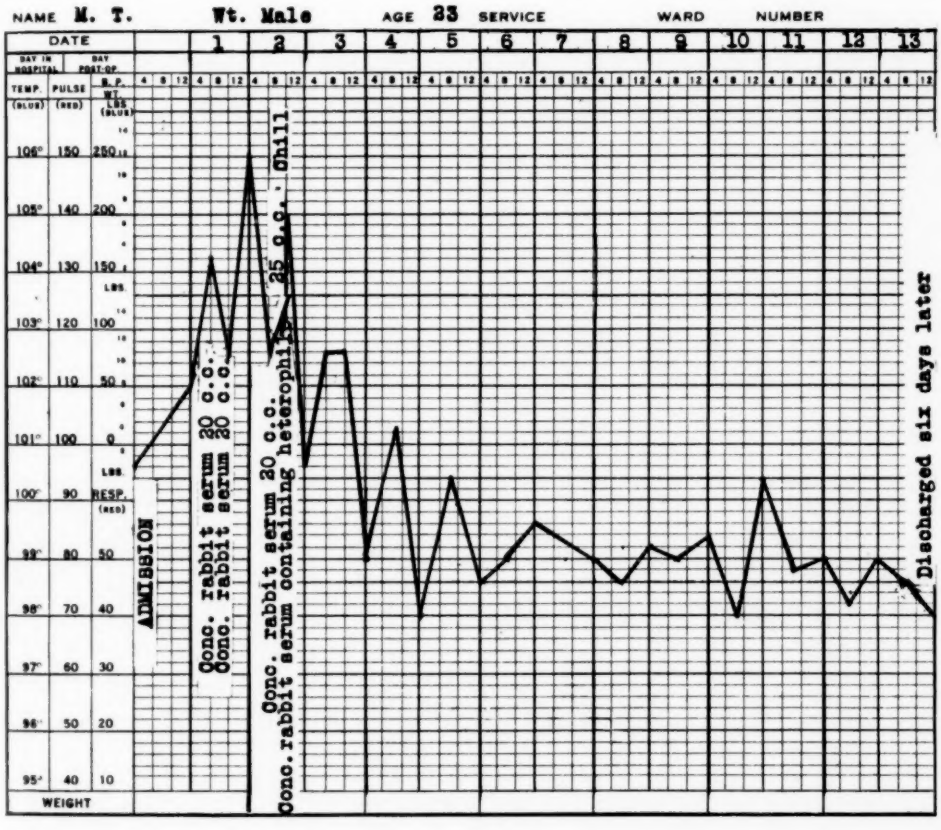

Chart 4.

From Oct. 25, 1933 to Apr. 13, 1934 we treated thirty-four additional patients. The results with these cases are shown in Table III.

If we consider all of the 68 cases together the results are shown in Table IV.

TABLE III

SHOWING RESULTS OF PNEUMONIA CASES TREATED WITH COMBINED HETEROPHILE ANTISERUM (OCTOBER, 1933, TO APRIL, 1934)

	NO.	DIED	RECOVERED	MORTALITY PERCENTAGE
Total	34	13	21	38.2
White	22	4	18	18.1
Black	12	9	3	75.0

NOTE: Of the white deaths one received serum 72 hours after onset, and the other three patients received their first serum 120 hours after onset. Of the 9 colored patients who died, 4 received their first serum over ninety hours after onset, 4 other patients died less than twenty-four hours after admission.

TABLE IV

SHOWING THE TOTAL RESULTS OF ALL OF THE PNEUMONIA CASES TREATED WITH HETEROPHILE ANTISERUMS (JANUARY, 1933, TO APRIL, 1934)

	NO.	DIED	RECOVERED	MORTALITY PERCENTAGE
Total	68	29	39	42.6
White	38	10	28	26.3
Black	30	19	11	63.3

From Table IV it is seen that 29 out of 68 patients died. But 11 of these patients did not receive their first antiserum until more than ninety hours had elapsed after the onset of the disease; 5 other patients died within twenty-four hours of their admission to the hospital, and 1 was a chronic alcoholic.

The heterophile antibody being theoretically effective on all four types of pneumococci, the clinical results of its uses on Types III and IV are interesting and are shown in Table V.

TABLE V

SHOWING THE RESULTS OF TREATMENT OF TYPES III AND IV WITH HETEROPHILE ANTISERUM

	NO.	DIED	RECOVERED	MORTALITY PERCENTAGE
Type III	10	4	6	40.0
Type IV	23	9	14	39.1

SUMMARY

We have given:

1. A brief discussion of the biologic importance of heterophile antibodies to pneumococcic pneumonia in man.

2. A description of the preparation of a pneumococcus antiserum containing heterophile antibodies.

3. The results of the treatment of sixty-eight patients having pneumococcic pneumonia with antiserum containing heterophile antibodies.

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ON SOME PRACTICAL CONSEQUENCES OF THE INFLUENCE OF TEMPERATURE UPON ISO-AGGLUTINATION*

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THERE are in the literature a few (approximately six) reports of the transfusion of incompatible blood in the usual quantity without a fatal outcome, and in some instances with hardly any reaction whatever. Two of these cases have been studied by staff members of the Donor Bureau of the Blood Transfusion Betterment Association, and in both instances the absence of symptoms was explained by the absence of agglutination of the donor's incompatible blood by the recipient's serum at body temperature.[1, 2] The question arises as to how often this might occur in similar circumstances.

Moreover, the observed lesser iso-agglutination at body temperature suggests that high-titered Group O donor bloods, which on a quantitative basis according to tests carried out at room temperature have been deemed dangerous for other groups, might be shown to be less dangerous if the tests were conducted at body temperature.

Actually, we have knowledge of one instance, observed in the early history of the Donor Bureau, in which a Group O donor, whose blood, on account of the unusually high titer of anti-A iso-agglutinins, had been designated as dangerous for transfusion into a Group A recipient, had given 500 c.c. of blood to a patient who later was identified by test as of Group A. Not the slightest reaction resulted from the transfusion.

We obtained blood from this patient and by the quantitative compatibility test (done at room temperature) we confirmed the impression that the transfused Group O serum should have caused a marked agglutination of the entire blood of the patient.

The study of these questions was taken up by determining the iso-agglutinating power of a number of Group A and Group B serums at room temperature with the usual method of serial dilutions; at the same time carrying out a single test at body temperature (about 40° C.) in a proportion of serum and cells with which a rough quantitative comparison could be made at the two temperatures, and with which also it could be seen whether at the body temperature the agglutination was absent or very slight. Table I represents an example of such a test.

The cells were used always in a 1-50 suspension, in a quantity of 4 capillary drops. The same capillary pipette was used for distributing the serum dilutions and the cell suspensions. The use of the capillary pipette for this

*From the Donor Bureau of the Blood Transfusion Betterment Association of New York City.

Received for publication, July 12, 1935.

purpose was important as a time saver especially in making the mixtures of the warmed solutions. In the instance cited in Table I the undiluted serum caused only a very slight agglutination at 40°, but a distinctly stronger agglutination with 1 drop of a 1-8 dilution at 17°. It could be estimated from this result that the agglutination was at least 32 times as strong at 17° C. as it was at 40°. The control mixture at 40° was important in aiding the detection of the slight degrees of agglutination and also in a few instances the absence of any agglutination at 40° C.

TABLE I

		WARM BATH 40°			COLD BATH 17°						CONTROL
			CONTROL T.								
Serum, undiluted		4 drops	4 drops	4 drops	2 drops	1 drop					4 drops
Serum, diluted	1- 2						1 drop				
	1- 4							1 drop			
	1- 8								1 drop		
	1-16									1 drop	
Incompatible cells	1-50	4 drops		4 drops	4 drops	4 drops	4 drops	4 drops	4 drops	4 drops	
Compatible cells	1-50		4 drops								4 drops
Agglutination		±	None	4+	3+	3+	2+	2+	1+	None	None

In order to maintain the "body temperature" mixture at temperatures not lower than 37° while the results were being read, all the tubes containing these mixtures were placed inside a somewhat larger tube containing 1 c.c. of warm water. This column of water filled roughly one-third to one-fourth of the space between the two tubes.

The mixtures for the tests at 40° were made after the serum and cells had been brought to that temperature, having stood in the warm bath for one hour. The capillary pipette used for distributing the cells was first cautiously warmed over a flame and the cell suspension was quickly added to the serums. All mixtures were shaken and allowed to stand in their respective baths for two hours. In those instances in which the clumping (with the undiluted serums) at 40° was 4-plus such serums were also tested at 40° C. in serial dilutions, to determine the approximate and relative agglutinating power.

The results were read with the naked eye, the mixtures being observed for this purpose in the tubes.

Forty-four Group A (anti-B serums) and 9 Group B (anti-A serums) were tested, 53 specimens in all. In Table II are shown the degrees of agglutination of the corresponding agglutinable cells by the 53 undiluted serums at 40° and 17° C.

It is seen that 3 of the serums caused no detectable clumping at 40° C. although they caused a 3-plus or 4-plus agglutination at 17° C. Two other serums caused only the slightest degree of agglutination at 40°, but 2-plus or

4-plus agglutination at 17° C. From these results it would seem that nearly 10 per cent of the serums exhibited so little agglutinating power as to make it seem likely that the transfusion of incompatible blood in the respective individuals might have been followed by slight symptoms or none at all. By making a rough comparison of the degree of clumping at the two temperatures, an estimate of the relative strength of the agglutination could be made as set forth in Table III.

TABLE II

DIFFERENCES IN THE AGGLUTINATION BY THE UNDILUTED SERUM AT 40° C. AND 17° C.

NUMBER OF SPECIMENS	AGGLUTINATION IN WARM BATH	AGGLUTINATION IN COLD BATH
2	None	3+
1	None	4+
1	±	2+
1	±	4+
3	1+	3+
5	1+	4+
5	2+	3+
17	2+	4+
16	3+	4+
2	4+	4+

TABLE III

NUMBER OF SPECIMENS	6	8	1	16	10	11	1
Times stronger in cold bath than in warm bath	64	32	24	16	8	4	2

The summary of these results in Table III shows that in every case the agglutination was stronger at room temperature than at body temperature. This relation has had frequent mention by other authors.

The serums of 24 "dangerous" Group O (universal) donors were studied in the manner described above. The criterion of dangerousness of such donors has been that one part of the donor's serum will produce distinct agglutination of 5 parts of A or B cells, respectively. These tests have been carried out at room temperature.

When the 24 serums were tested at 40° C. in serial dilutions, only 11 were found capable of producing distinct agglutination of 5 parts by volume of the packed cells of the respective incompatible blood. These 24 donors were derived from a group of about 350 Group O donors. If on a mathematical basis we accept as actually dangerous the 11 whose serums cause distinct agglutination of 5 parts of packed incompatible cells at 40° C., this would give a percentage of approximately 3. When it is considered that other tissues besides the blood elements are capable of taking up the transfused isoagglutinin of the dangerous universal donor, it seems probable that transfusions could be given by at least some of these 11 into Group A or Group B patients without causing symptoms.

The result of this study seems to justify the carrying out of the quantitative serum titration for the detection of the so-called dangerous universal donor at the body temperature.

SUMMARY

1. About 10 per cent of undiluted Group A and Group B serums cause only slight clumping of the corresponding agglutinable cells, or apparently none at all, at body temperature (40° C.). Transfusion of incompatible blood into such patients might be expected to be followed by only mild symptoms or none at all.

2. The quantitative tests used in the detection of the "dangerous" universal donor may be properly carried out at body temperature, the incidence of the "dangerous" universal donor being estimated by this method at approximately 3 per cent of Group O individuals.

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THE OXYGEN ABSORBING POWER IN THE PRESENCE OF CERTAIN DISEASES*

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THE amount of oxygen absorbed for each unit of oxygen respired can readily be calculated from a basal metabolic rate chart as has been shown by Ziegler.[1] The percentage of respired oxygen utilized was termed by Ziegler as the "oxygen absorbing power."

The amount of oxygen respired by an individual is calculated by multiplying the respiratory rate by the respiratory amplitude. The amount of oxygen consumed divided by the amount of oxygen respired gives the absorption-respiration ratio, or the oxygen absorption power as given by the following formula:

$$\text{Oxygen absorption power} = \frac{\text{O}_2 \text{ consumption per minute (uncorrected)}}{\text{O}_2 \text{ respired per minute (uncorrected)}} \times 100$$

We studied a group of 366 cases which was unlike Ziegler's in that both males and females of greatly varied ages and condition were included, whereas his study of 160 cases was restricted entirely to relatively healthy males. His first group of cases consisted of young men from the United States Citizen's Conservation Corps who entered the hospital only for the treatment of major or minor surgical conditions. His second group consisted of veterans of the World War. These had various major or minor chronic diseases for the most part, but the majority were convalescing from disabilities received in the war.

Ziegler found that the average oxygen absorbing power was 4.0 per cent, varying from 3.9 per cent in individuals under thirty years of age to 4.1 per

*From the Cleveland Clinic.
Received for publication, July 26, 1935.

cent in persons in the age group above thirty. This age difference is probably insignificant. His point of view is well summarized in the following paragraph:

"In the field of applied physiology, it appears that the oxygen absorbing power will be of great value. The higher the oxygen absorbing power the greater the ability to absorb oxygen from the available supply. The athlete with the highest index would have the best 'wind.' The diver with the higher index would be able to 'stay under' longer. The aviator with the highest oxygen absorbing power could probably go to higher altitudes, and the same would be true for the mountain climber."

We have investigated the oxygen absorbing power of a number of patients who suffered from various disorders, in order to determine whether this measurement under basal conditions is indicative of the respiratory efficiency of the same individual under other conditions.

Since most of the basal metabolic rate studies in our records were made on patients in whom disease of the thyroid gland was suspected, we first wished to determine whether the oxygen absorbing power varied with the amount of oxygen being utilized. We therefore calculated the oxygen absorbing power in a group of 80 patients with basal metabolic rates which varied from minus 30 per cent to plus 81.1 per cent. None of these patients suffered from pulmonary or cardiovascular disorders, nor were any of them known to have anemia.

The total average oxygen absorbing power of patients with basal metabolic rates below normal was 4.23 S. D. $\pm$ 0.106, and for those above normal it was 4.48 S. D. $\pm$ 0.093. The average oxygen absorbing power of patients in both these groups above thirty years of age was 4.36 per cent, and for those below this age limit 4.40 per cent. These figures are close to the average values found by Ziegler. From these findings, we concluded that the oxygen absorbing power under basal conditions was not influenced significantly by age, sex, or metabolic rate.

In studying these two groups, it was interesting to note that certain weak, middle-aged individuals apparently had an oxygen absorbing power equal to that of young, robust patients. This fact would lead one to believe that the oxygen absorbing power under basal conditions is not a measure of the respiratory efficiency during activity.

One might expect that the oxygen absorbing power would be a function of the amount of hemoglobin in the blood. We therefore investigated a group of 50 individuals who were suffering from anemia, and as shown on Table I, the oxygen absorbing power of this group of patients was found to be high. These patients were those who, under normal conditions of activity, were obviously low in respiratory efficiency. It therefore seems that the oxygen absorbing power under basal conditions is not a measure of the respiratory efficiency during activity. The explanation of this probably lies in the fact that in anemia, compensatory mechanisms come into play which permit of a certain amount of activity, but result in overcompensation when the patient is at rest.

Dautrebande[2] has shown that the cardiac output in anemia is definitely increased. His results show that when the hemoglobin percentage is decreased

from 100 to 50 per cent, the output of the heart is practically normal, but when the hemoglobin percentage is lower than 50 per cent, the output of the heart increases very rapidly.

TABLE I

OXYGEN ABSORPTION POWER IN THE PRESENCE OF CERTAIN DISEASES

	HIGH BASAL METABOLIC RATE		LOW BASAL METABOLIC RATE		ANEMIA	CARDIOVASCULAR DISEASES	PULMONARY DISEASES
	Men	Women	Men	Women			
Number of observations	16	34	10	20	50	51	49
Highest value	5.60	5.50	5.57	5.11	9.18	10.06	4.72
Lowest value	3.55	2.61	3.38	3.17	3.84	3.42	2.26
Average	4.55	4.40	4.39	4.20	5.23	5.15	3.64
Standard deviation from the mean	0.15	0.117	0.178	0.142	0.153	0.154	0.0707
Standard deviation of a single observation	0.189	0.215	0.1784	0.2005	1.323	1.08	0.4960

Hurtado, Kaltrieder, and McCann,[3] in their experiments on respiratory adaptation in anoxemia, found that the respiratory response to anoxemia included structural changes in the lungs which consisted chiefly of a widening in the capillaries and a dilatation of the alveoli. These changes were accompanied by a moderate increase of the residual air, and a corresponding decrease in the vital capacity, with little or no change in the pulmonary capacity. There appears to be sufficient evidence to suggest that these changes are compensatory in character. They tend to produce an increase in the surface for diffusion between the circulating blood and the alveolar air, a condition favorable for a more efficient exchange of the respiratory gases, and therefore a higher oxygen absorbing power.

Liljestrand and Stenström[4] found that in individuals suffering from anemia, the proportion of oxygen in the blood that is utilized by the tissues during rest is greater than in normals. This enables the patient with anemia to maintain the combustion processes during rest with only a moderate increase in the blood flow and the work of the heart.

In a few of the patients whom we examined, the compensatory changes had not been sufficient to make the oxygen absorbing power normal even under resting conditions.

An examination of a series of 51 patients who suffered from cardiovascular lesions showed that the compensatory respiratory changes which occur in diseases of the heart are usually such that the oxygen absorbing power of the patient at rest is greater than that of normal individuals (Table I).

In a group of 49 patients who were suffering from pulmonary disorders, the average oxygen absorbing power was found to be below normal, and the findings (Table I) in this group of patients are in definite contrast to those in the group suffering from cardiovascular diseases or from anemia. The explanation for this is that there appears to be no compensatory mechanism which enables this type of individual to absorb the normal percentage of oxygen inhaled while at rest.

This test obviously is not a measure of the respiratory fitness of individuals for athletic and other endeavors. If the selection depended on these tests, individuals might be chosen who are suffering from a grave cardiovascular disorder or anemia.

The question arose as to whether or not the routine calculation of the oxygen absorbing power of all individuals on whom basal metabolic rates were measured would be of value in discovering undiagnosed cases of anemia or cardiovascular diseases. In this study, 106 patients who were selected at random were investigated. Of this group, thirteen had an oxygen absorbing power over 5.5 per cent. These individuals were found to have either a cardiovascular disorder or anemia. In each case, however, the clinician in charge had been aware of the condition before the basal metabolic rate determination was made. We therefore concluded that the routine calculation of this index was of little value.

SUMMARY

A study of the oxygen absorbing power of patients under basal conditions shows that it is not a measure of the respiratory efficiency of these individuals while they are active.

The oxygen absorbing power during rest is influenced but slightly, if at all, by changes in thyroid activity.

The oxygen absorbing power usually is normal or above normal in patients suffering from anemia or cardiovascular disorders. In case of patients suffering from pulmonary disorders, the basal respiratory efficiency is usually normal or subnormal.

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RECENT ADVANCES IN ENDOCRINE DIAGNOSIS AND TREATMENT*

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WHILE endocrine disturbances frequently confront every doctor, not every patient should be subjected to an endocrine survey. The following conditions should lead to an endocrine examination:

1. Cases where the diagnosis is uncertain and not well supported by clinical and laboratory data.

2. Cases where the diagnosis seems correct but the indicated treatment fails to produce the results the doctor has reason to expect.

3. Every case of neurasthenia and nervousness and cases where there is uncertainty as to whether the patient has Graves' disease. While this syndrome is undoubtedly due to some endocrinopathy beyond the thyroid and probably will shortly be removed from the list of surgical diseases, at this time most men should merely make certain that there is not some other endocrinopathy responsible for the patient's complaints.

4. Growth deficiencies.

5. Genital hypoplasia.

6. Menstrual disorders not clearly due to local pathology in the pelvis.

7. Headaches.

8. Obesity.

9. Certain bone conditions, such as cysts, decalcification, slipping of the epiphyses, and fractures that fail to unite.

10. Diabetes mellitus.

11. Essential hypertension.

12. Patients who complain of mental or physical retardation, loss of memory and lack of concentration.

The diagnosis of endocrinopathies should be based on:

1. The exclusion of nonendocrine conditions.

2. A detailed history of the patient's complaints and of their relation to heredity, infection, surgical operation, or other trauma; also their relation to menstruation and pregnancy. The patient's developmental history should show, if possible, the weight at birth, whether the patient was delivered at full term, whether there was a likelihood of birth injury, whether the patient was bottle fed, the presence or absence of gastrointestinal upsets, and the age at which the teeth appeared and when the patient began to walk and talk. It is helpful also to know the course of the pregnancy at the termination of which the patient was delivered.

The menstrual history should disclose the age at onset, the interval between periods, the duration and amount of the flow and whether it has changed materially as to amount or duration; the location of pain and the time of occurrence, before, during or after the period; whether headaches are present and their relation to the periods: pituitary headache frequently occurs a few days before

*Delivered before the Peoria City Medical Society, June 18, 1935.
Received for publication, July 2, 1935.

the period; and whether the patient is the victim of colds at each menstrual period. If the patient has been pregnant, it is helpful to know the course of the pregnancies and how they terminated, whether by miscarriage or at full term. Habitual abortion is due to some endocrinopathy quite as often as it is to syphilis. One should also inquire whether the mother was able to nurse her children. Lactation is now known to be influenced by the anterior pituitary, and victims of anterior lobe deficiency frequently are unable to nurse their babies.

The history should also inquire into the height and weight of the relatives.

Physical Findings: In addition to the routine careful physical examination, one should pay particular attention to the patient's stature, the proportion of the upper to the lower measurement and the relation of the span to the height, and to the condition of the skin and its appendages, the hair, nails and teeth. If obesity is present, its location should be noted, and the history should show whether it was acquired after an infection, such as measles or typhoid, after a surgical operation or after pregnancy. Pituitary obesity occurs under such circumstances.

Whether the anterior lobe makes as many hormones as it is sometimes credited with is doubtful; but it does elaborate a growth and a sex hormone between which there appears to be some antagonism, so that if the growth hormone is produced in superabundance, it may be at the expense of the sex hormone and vice versa. A youngster lacking a sufficient amount of the sex hormone may not only exhibit hypoplastic genitalia but also an increased growth of the long bones. This gives rise to the eunuchoid type of individual whose lower measurement (from symphysis to sole) may exceed by several inches the upper measurement (symphysis to vertex) and whose span may exceed the height. Other individuals lack the growth hormone but have a superabundance of the sex hormone. This results in well-developed genitalia with a lack of growth of the long bones—an individual with short legs and a long body, whose lower measurement may be much shorter than the upper.

Laboratory Data.—In some instances this may need to be extensive. In others but little may be required. Most endocrinopathies exhibit lymphocytosis. This is most marked in thyroid deficiency. An increase in the eosinophiles is commonly present in pituitary deficiency and is less marked in adrenal and ovarian insufficiency. The blood sugar is usually low in pituitary deficiency, and the glucose tolerance curve is usually low, returning to the fasting level or slightly below at the end of four hours. The urea is said to be increased in adrenal insufficiency except late in Addison's disease. This is probably not often well enough marked to be very helpful. The uric acid is usually increased in pituitary deficiency. The calcium gives some help in studying the level of parathyroid function.

The x-ray is helpful in determining the bone age and the presence of bone cysts and of decalcification in hyperparathyroidism or pituitary basophilism. In studying bone age, various joints need to be x-rayed at different ages. The x-ray is one of our most useful implements in deciding whether a child under six is a victim of thyroid deficiency at ages when the basal metabolic rate gives unreliable information.

The visual fields are frequently contracted in the presence of pituitary deficiency.

Maliner's test consists of the injection of 1 c.c. of adrenalin. This is said to accentuate heart murmurs, to localize them more definitely to the areas over which they should be heard, and to develop murmurs not usually heard.

The insulin test was suggested by Harrop and others for a study of early or mild Addison's disease. The intravenous injection of very small doses of insulin is followed in such cases by prolonged and sometimes violent hypoglycemic attacks, which respond very indifferently to glucose either by mouth or by vein. The doctor who contemplates using this test should have his patient in the hospital.

Withholding table salt from the patient may bring about considerable aggravation of his symptoms if they are due to adrenal insufficiency.

The basal metabolic rate is always helpful. We have, unfortunately, believed that it reflected only thyroid function; that a low basal metabolic rate was caused or accompanied by a low thyroid function; but both pituitary and adrenal insufficiency may be responsible for very low basal readings. If a patient is found to have a low basal metabolic rate and few signs of thyroid deficiency, a search should be made for signs of pituitary or adrenal insufficiency. This is particularly true when the patient cannot tolerate even moderate doses of thyroid.

The signs and symptoms of hypothyroidism are too well known to need repetition here. It is an extremely common condition. It is best treated by the use of desiccated thyroid rather than by thyroxin for the reason that the body's response to thyroid can be more nearly controlled. It should be remembered that thyroid increases the heart's work before it increases its strength so that patients with severe degrees of thyroid deficiency are apt to feel worse after treatment is instituted. They may experience a good deal of palpitation, precordial distress, nervousness, and a trembling feeling. In such instances the thyroid should be stopped for a few days and then resumed in smaller doses or the patient should be put to bed for a few days.

Some relatively new endocrine syndromes have been described. In 1932 Cushing[1] described pituitary basophilism. This is characterized by vascular hypertension, hyperglycemia, frequently glycosuria and many times hypercalcemia. There is a rapid gain of painful obesity which is limited largely to face and trunk. There is an increase in hair growth. The menstrual periods usually cease. Flame-shaped purplish lineae atrophicae are noted in the groin, ecchymotic spots appear on the legs and the skin becomes peculiarly mottled. Frequently the patient complains of backache and pains in the joints, precordial distress, and difficulty in breathing. Wilder and others[2] have reported similar syndromes associated with adenoma of the adrenal cortex and so the picture is not so distinct as was thought at first.

Hyperparathyroidism is usually due to tumor or hyperplasia of one or more of the parathyroids. It is characterized by a disturbance of calcium metabolism. Calcium is usually present in large amounts in the blood stream and is excreted in large amounts in the urine. It is abstracted from the bones with their resultant rarefaction and softening. Cysts and tumors frequently occur. The

victim decreases in height and sometimes becomes a twisted gargoyle-like caricature of his former self. Calcium deposits in the kidneys are common, polyuria and polydipsia occur, and much pain is complained of in the bones and joints. Treatment consists in removal of the tumor or one or more hyperplastic parathyroids.

Hyperinsulinism was first described by Seale Harris.[3] Its symptoms are those of insulin shock. Epileptoid seizures frequently occur, and many patients have been diagnosed as epileptics. A low blood sugar is found during these attacks. There is doubt in my mind that hyperinsulinism is always at fault in these syndromes, although a few well-authenticated cases are on record where a pancreatic tumor was found and removed with cure of the syndrome. In other cases, however, the patient was best relieved by doses of pituitary or suprarenal by mouth or hypodermically. At autopsy the pancreas was found to be a third the normal size and architecturally appeared to be normal. Even if the doctor prefers to call these cases hyperinsulinism, he should at least look for pituitary or adrenal deficiency.

Adrenal hemorrhage has been thought of as something recognized either at the autopsy table or shortly before the patient arrived at that point. However, Goldzieher and Gordon[4] feel that adrenal hemorrhage in infancy should be recognized during life and that many patients can be successfully treated. The picture they draw of this condition is that of pneumonia plus a petechial rash on some part of the body, usually the extremities, and without the lung findings characteristic of pneumonia.

Adrenal Hypertension.—It seems now fairly definitely established that some cases of paroxysmal hypertension are associated with tumors of the adrenal medulla or of similar tissue situated elsewhere in the body. Constant hypertension is said to be more often associated with hyperplasia or tumor of the adrenal cortex.

New Hormones.—Preparations of the sex and growth hormone of the pituitary are now available. The experimentalists also use the thyrotropic, the adrenotropic, the diabetogenic and the fat metabolism hormone from the anterior lobe. Riddle and his workers have isolated a substance from the anterior lobe which is influential in lactation. They call it prolactin. Injected into laboratory animals it not only brings about lactation but also changes the emotional reaction so that virgin rats manifest maternal instincts. A pigmentation hormone is claimed for the pars intermedia. The testicular hormone has been isolated and identified.

New Therapeutic Agents.—The parathyroid hormone is now available commercially. It is well standardized. A preparation of the hormone of the adrenal cortex essential to life is marketed under the name of eschatin. It should not be forgotten, however, that the adrenal cortex probably elaborates other hormones. At any rate the adrenals are concerned in the metabolism of sulphur, calcium, pigment, and sodium, tissue respiration and carbohydrate metabolism.

An insulin-free extract of the pancreas is marketed under a number of names: padutin, angioxyl, and kallikrein. It is useful in the treatment of angina pectoris. It has some value in the relief of pain and in increasing the blood flow through the extremities in various circulatory disorders. It is said to be useful in essential hypertension.

The sex hormone of the anterior lobe, or something that closely resembles it, can be separated from the urine of pregnant women and also from the placenta. Preparations of it are marketed under the trade names antuitrin-S, follutein, A.P.L. and progynon. These are useful in the treatment of amenorrhea, oligomenorrhea, and menorrhagia. For scanty or absent periods small doses are used; for the control of functional uterine bleeding larger doses are necessary, 2 or 3 c.c. daily. These preparations are of no value in the treatment of menopausal disorders. They are extremely valuable in the treatment of frigidity and sexual infantilism. They are also effective in the treatment of cryptorchidism.

Diathermy is recommended in the treatment of menstrual disorders associated with thickening of the ovarian tunic. The functional level of the adrenals and the pancreas is said to be raised by diathermy applied to those regions.

The parathyroid hormone is specific in the treatment of parathyroid tetany. It is also useful in the treatment of some forms of colitis, particularly the mucous variety, and in a syndrome which closely resembles, if it is not identical with, sprue. The French claim to have noted improvement in peptic ulcer following its use, and some cases of arthritis have been freed from pain by it. Some gynecologists report the control of menorrhagia by its use, and some cases of urticaria are relieved by it.

Various preparations of the ovarian hormone are available, such as theelin, amniotin, theelol, etc. These are particularly useful in disturbances of the menopause, not only the vasomotor phenomena but also the gastrointestinal complaints and the mental and emotional upsets occurring in this connection. At the Elgin State Hospital we treated seventeen women suffering from ovarian insufficiency. Ten of these improved to the point where they were able to leave the institution. Larger series with as good or better results have been reported by other men.

While the testicular hormone has been isolated, preparations of it are not available for the clinician. Probably the nearest approach to it is the testicular emulsion made by Stanley. It is not marketed but through the kindness of Dr. Stanley we were supplied with enough to treat three dementia precox cases in the Elgin State Hospital. Two of these very promptly improved to the extent that they were able to leave the hospital. Undoubtdely there is a close relationship between emotional and mental stability and the supply of the gonadal hormones.

For some years so-called light or stimulating doses of the x-ray have been applied to the pituitary and ovaries for the treatment of amenorrhea, dysmenorrhea, and menorrhagia. Many of these conditions were relieved and no bad effects were reported. Later Cushing used it in a few cases of pituitary basophilism. More recently we have used it in the treatment of diabetes mellitus and essential hypertension. Of hypertension alone we have treated 110 men and 54 women. Of these, 62 men and 35 women experienced some improvement. Nineteen men and 6 women were not improved. Twenty-five men and 8 women had an insufficient amount of treatment and 4 men and 5 women could not

be followed. We have had 18 patients who had both hypertension and diabetes, 13 men and 5 women. Of these, 7 experienced improvement in both conditions; in 4 the hypertension alone was improved without influencing the diabetic condition; in 3 the hypertension was not affected but the diabetes was improved; the others either had an insufficient amount of treatment or could not be followed.

Barnes,[5] working in Carlson's laboratory, removed the pancreas from ten dogs. These totally diabetic dogs, after being adjusted as to their diet and insulin requirement, were subjected to x-ray treatment applied to the pituitary and adrenals, following which their insulin requirement declined 50 per cent. These dogs were treated in pairs and the results in each pair were practically identical with those in every other pair.

Believing that O'Hare[6] was right in suggesting that patients exhibiting a diabetic type of sugar curve during a glucose tolerance test are victims of essential hypertension, we have performed 63 tests on 61 hypertensive patients. Forty-one of these patients exhibited a rise in blood sugar to above 170. Glycosuria was rarely observed. Twenty-nine of these were improved by treatment. However, of the 20 patients exhibiting a nondiabetic type of curve, 16 were improved. Therefore we have given up the idea that the glucose tolerance curve is of any prognostic value in this work.

In the beginning we used very small doses of the x-ray consisting of the following factors: 110 KVM, 10 × 10 cm. portal, 1 mm. aluminum and 0.25 mm. copper filter, 50 cm. skin target distance, 4 milliamperes and twenty minutes to each side of the pituitary and the adrenals. We were unable to escape the idea that if little is good more is better and so the dosage was gradually increased until in a few instances we used the following factors: 180 KVM, 10 × 10 cm. portal for each side of the pituitary, 15 × 15 cm. common portal for the adrenals, 1 mm. aluminum and 0.25 mm. copper filter, 50 cm. skin target distance, 5 milliamperes, fifteen minutes to the pituitary and twenty minutes to the adrenals, giving 142.5 r. units to the pituitary and 190 to the adrenals. Almost invariably patients experienced an unpleasant reaction to this dose, consisting of headache, tinnitus, weakness, and sometimes nausea. In only one case was there any notable reduction in blood pressure. We are convinced that whatever good can be accomplished can be brought about by very small doses of the ray. Consequently we returned to the small dose and now use the following factors: 120 KVM, 10 × 10 cm. portal for each side of the pituitary, 15 × 15 cm. common portal for the adrenals, 2 mm. aluminum filter, 50 cm. skin target distance, 4 milliamperes, seven minutes and about 70 r. units.

In the beginning treatment was given at intervals of once a week until six treatments were given unless the blood pressure returned to normal levels before this series was completed. Now if there is a substantial reduction in blood pressure after the first treatment, no further exposure is ordered until it is evident that the blood pressure will not decline further or until it begins to rise. Instead of an immediate fall in blood pressure after treatment, there is frequently a rise lasting seventy-two hours and then a considerable drop in pressure.

Patients should be seen frequently while they are under treatment. The roentgenologist should not be saddled with the entire responsibility for the treatment of these patients, but he should be certain that his machine is accurately calibrated so that he knows how many R units per minute are delivered by it. The doctor in charge should decide when treatment should be repeated and whether the dose should be increased or decreased. How permanent the results effected by this method of treatment are is a question yet to be answered.

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LABORATORY METHODS

A MODIFICATION OF THE GREENBERG TECHNIC FOR THE COLORIMETRIC DETERMINATION OF SERUM PROTEIN*

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VARIOUS colorimetric procedures have been described for the determination of serum proteins.[1, 2, 3] These methods are all based on the assumption that under the experimental conditions described there is a constant relationship between the amount of protein present and the chromogenic value of a given serum or solution of protein as measured by the molybdate-tungstate reagent (phenol reagent). Although other chromogenic substances undoubtedly enter into the reaction the principal phenolic groups measured are presumably those of the tyrosine present in the protein molecule. The color obtained from a known amount of pure tyrosine is used for comparison and the chromogenic value is usually expressed as tyrosine.† Provided that the assumed constant relationship holds, the amount of any protein for which the tyrosine: protein ratio has been established can be calculated from the results obtained from the colorimetric determination of the tyrosine present. The wide discrepancies in the protein equivalents of 1 mg. of tyrosine established by the various investigators quoted above indicate that the experimental conditions of the color development greatly influence the color obtained. This fact, however, would not necessarily preclude a constant relationship provided that under the prescribed conditions the liberation of chromogenic groups proceeded always to a definite and permanent end-point.

The chief advantage offered by the modification of Greenberg[3] over the older colorimetric procedures was the introduction of the use of the improved phenol reagent of Folin and Ciocalteu.[4] This reagent avoided the difficulties of turbidity which had been previously encountered and made it possible to adopt a procedure which led to a rapid development of a more intense color from a given amount of protein. The technic described by the earlier investigators was further simplified by the adoption of Howe's[5] method for the fractionation of serum proteins. The procedure for production of the color as described by Greenberg required only the addition of 2 c.c. of 5 N sodium hydroxide to about 25 c.c. of a definite dilution of serum or of the albumin fraction at room temperature. Chromogenic groups were immediately made

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Received for publication, July 10, 1935.

This work was aided by a grant from the Division of Medical Sciences of the Rockefeller Foundation.

†For convenience throughout the rest of this paper we shall use, without further qualification, the term tyrosine to indicate the chromogenic value of a serum or protein fraction as measured colorimetrically by the phenol reagent.

available to produce a deep blue color with the phenol reagent. By making the resulting colored solution to a definite volume and comparing with the color similarly derived from a known amount of tyrosine, the tyrosine value of the unknown was determined. From a comparison of his colorimetric results with the protein content of the same serums as determined by the Kjeldahl method, Greenberg derived factors which, under the described conditions, were assumed to hold generally for the calculation of the various protein fractions from their tyrosine content.

An attempt by us to apply this procedure and these tyrosine: protein factors to the routine determination of serum proteins gave results which at times varied widely from the figures obtained on the same serum by the Kjeldahl method. Our colorimetric results were at times too high and at other times too low. An investigation of the cause of these discrepancies convinced us that we were dealing with a rapidly progressing and, under the prescribed conditions, always incomplete reaction between the protein solution and the sodium hydroxide. Both the temperature of the solution at the time the sodium hydroxide was added and the time allowed to elapse between the addition of the sodium hydroxide and the phenol reagent greatly influenced the amount of color developed from the same amount of a given serum. The longer the time allowed for the reaction with sodium hydroxide and the higher the temperature at which it was carried out the greater the amount of color developed. These factors of time and temperature had so great an influence that even the range in room temperature encountered in this climate during a year made a great difference in the results obtained. Similarly at a given temperature the time allowed made so much difference that even a slight delay in adding the phenol reagent significantly increased the amount of color developed. In Table I are presented data which show the gradual increase in tyrosine values obtained on the same serum as the time allowed for the hydrolysis with sodium

TABLE I

EFFECT ON YIELD OF TYROSINE OF VARYING DURATION AND TEMPERATURE OF HYDROLYSIS WITH SODIUM HYDROXIDE*

TEMPERATURE ° C.	MG. TYROSINE PER 100 C.C. SERUM				
	2 MIN.	5 MIN.	10 MIN.	20 MIN.	30 MIN.
20°	426	444	468	482	503
25°	450	470	494	510	513
30°	460	482	510	519	530
35°	465	488	513	533	533
40°	491	516	530	540	548
45°	503	537	556	563	563
50°	522	556	560	580	584

*0.2 c.c. of serum + 20 c.c. H_2O + 4 c.c. 10 per cent NaOH used in every case. Same serum throughout.

hydroxide was lengthened at increasing levels of temperature. The results show that even after thirty minutes at 50° C. the chromogenic value is still increasing quite rapidly. Obviously no constant results could be obtained until conditions were worked out which would bring the reaction to a definite and constant end-point. To accomplish this the treatment with sodium hy-

droxide was carried out in flasks immersed in a briskly boiling water-bath. Although a strongly positive biuret reaction shows that the protein has not been completely hydrolyzed by this procedure, the figures presented in Table II indicate that a constant chromogenic value has been reached. On the five samples of serum studied the tyrosine values were constant after heating for periods from five minutes to one hour. Based on these observations the following procedures were adopted for the colorimetric determination of tyrosine in serum protein.

TABLE II

EFFECT ON THE YIELD OF TYROSINE OF CARRYING OUT HYDROLYSIS FOR VARYING LENGTHS OF TIME IN BOILING WATER-BATH*

SERUM NUMBER	MG. OF TYROSINE PER 100 C.C. SERUM					
	5 MIN.	10 MIN.	15 MIN.	20 MIN.	30 MIN.	60 MIN.
1	580	576	576	571	–	–
2	549	544	544	541	549	549
3	479	479	482	479	482	482
4	537	537	533	537	537	537
5	584	592	588	592	592	584

*0.2 c.c. serum + 20 c.c. H_2O + 4 c.c. 10 per cent NaOH used in every case.

Total Protein.—A one to ten dilution of the serum was made with physiologic saline. Two cubic centimeters of this dilution representing 0.2 c.c. of the original serum were placed in a 50 c.c. volumetric flask. Twenty cubic centimeters of water and 4 c.c. of a 10 per cent solution of sodium hydroxide were added. The flask was then placed in a boiling water-bath for ten minutes. After cooling the contents of the flask thoroughly by standing it in ice water 3 c.c. of phenol reagent (Folin and Ciocalteu[4]) were added and the volume made to 50 c.c. with distilled water. A standard containing 0.8 mg. of tyrosine in a volume of 50 c.c. was prepared in a similar manner. After allowing ten minutes for the color to develop comparison was made in a colorimeter. The standard tyrosine solution need not be heated with the alkali as no difference in the amount of color derived from pure tyrosine results from the procedure. For pathologic serums less concentrated standards containing 0.6 mg. or even 0.4 mg. of tyrosine may be necessary for satisfactory colorimetric comparison. When the usual 0.8 mg. standard is used and the standard color is set at 20 mm. in the colorimeter the calculation becomes:

$20/R \times 400 =$ mg. tyrosine per 100 c.c. R = the reading of the unknown solution.

Even with the improved phenol reagent containing lithium sulphate we have noted a slight tendency for the solution to cloud if the laboratory temperature is very high, unless the flasks are kept in ice water during the ten-minute interval allowed for color development. Even a barely perceptible cloud influences the colorimetric readings in the direction of giving too high results.

Attention should be called at this point to the addition of water to the diluted serum before the sodium hydroxide is added. This was done in the determination of total protein and of albumin in order to make the dilution of alkali for the hydrolysis the same as was later found inevitable for globulin.

It was found impractical to add larger amounts of sodium hydroxide to the protein dilutions because of the greater tendency for the colored solutions to cloud when the salt content was much increased. The 4 c.c. of 10 per cent NaOH which we have used is equivalent to the 2 c.c. of 5 N NaOH used by Greenberg.[3]

Serum Albumin.—The albumin and globulin fractions were separated by salting out the globulin with sodium sulphate according to the method described by Howe.[5] The serum was diluted by adding twenty volumes of 22.5 per cent sodium sulphate. This results in a 1:21 dilution of the serum and a final concentration of 21.4 per cent sodium sulphate. After the addition of sodium sulphate the mixture was allowed to stand for several hours, usually overnight, in an incubator at 38° C. The globulin was then removed by filtration and the tyrosine content of the filtrate which contains the albumin was determined. Five cubic centimeters of the filtrate were placed in a 50 c.c. volumetric flask and 15 c.c. of water and 4 c.c. of 10 per cent NaOH added. The heating with the alkali and the color development with phenol reagent were then carried out exactly as has been described in the case of total protein. A standard containing 0.8 mg. of tyrosine in 50 c.c. gives a color suitable for comparison with the tyrosine obtained from the albumin of normal serums. More dilute standards may be necessary for pathologic specimens. The calculation using the 0.8 mg. standard set at 20 mm. is:

$$20/R \times 0.8 \times 2100/5 = \text{mg. tyrosine per 100 c.c.}$$

This may be simplified to

$$20/R \times 336 = \text{mg. tyrosine per 100 c.c.}$$

The amount of tyrosine obtained from the globulin in 0.5 c.c. of serum gives a color suitable for colorimetric reading. For the determination of globulin 0.5 c.c. portions of serum were diluted with 10 c.c. of a 22.5 per cent solution of sodium sulphate and allowed to stand as described above. The globulin was removed by filtration, washed with the Na_2SO_4 solution to remove all the albumin, and dissolved and washed into a 50 c.c. volumetric flask with approximately 0.01 N sodium hydroxide. The amount of solution necessary for washing was inevitably about 20 c.c. and this amount was always used. To this solution was added 4 c.c. of 10 per cent NaOH and the determination of tyrosine was carried out exactly as has been described. Again the 0.8 mg. tyrosine standard is usually suitable and when this is set at 20 mm. in the colorimeter the calculation is:

$$20/R \times 160 = \text{mg. tyrosine per 100 c.c.}$$

Before these values of tyrosine could be used for the determination of protein the tyrosine: protein relationship had to be established for these various types of protein under the newly adopted experimental conditions. To establish these ratios 25 samples of human serum have been studied. The tyrosine values of the various fractions were determined by the procedures

described above. For comparison with these results the proteins were determined on the same samples by the macro-Kjeldahl method. The nonprotein nitrogen content of each serum was determined by the Folin and Wu method[6] and this amount subtracted in the case of total protein and albumin from the total nitrogen determined by the Kjeldahl method. No nonprotein nitrogen is carried over into the globulin fraction so no correction is made in that determination. The protein nitrogen value was converted to protein by the generally accepted factor of 6.25. The tyrosine: protein factor for the different types of protein was determined by dividing the milligrams of protein per 100 c.c. by the milligrams of tyrosine per 100 c.c. Our results obtained on the 25 serums are presented in Table III.

It will be noted that in some instances the globulin factors were determined only by difference, while in the remainder of the samples they were determined

TABLE III

DETERMINATION OF TYROSINE PROTEIN FACTOR

SERUM	TOTAL			ALBUMIN			GLOBULIN		
	MG. PER 100 C.C.		FACTOR	MG. PER 100 C.C.		FACTOR	MG. PER 100 C.C.		FACTOR
	PROTEIN	TYROSINE		PROTEIN	TYROSINE		PROTEIN	TYROSINE	
1	6300	497	12.67	3631	278	13.06	2669	219	12.19
2	7137	550	12.97	4481	356	12.59	2656	194	13.69
3	7025	543	12.94	4763	362	13.16	2262	181	12.50
4	7237	560	12.92	4750	366	12.98	2487	194	12.82
5	5476	440	12.45	3056	231	13.23	2420	209	11.60
6	7069	532	13.29	4720	358	13.18	2349	174	13.50
7	6833	510	13.39	4706	352	13.36	2127	158	13.47
8	7094	532	13.33	4700	352	13.35	2394	180	13.30
9	7257	537	13.51	4770	354	13.47	2487	183	13.58
10	6694	505	13.25	4806	342	14.05	1888	163	11.59
11	7175	518	13.85	4612	333	13.85	2563	185	13.85
12	7094	526	13.48	4861	350	13.88	{2756*	184*	14.98*
							{2233	176	12.66
13	7206	525	13.72	5156	380	13.57	{2225*	150*	14.83*
							{2050	145	14.13
14	7446	569	13.08	4993	367	13.60	2453	202	12.15
15	6169	476	12.96	3125	234	13.35	{3156*	248*	12.76*
							{3044	242	12.58
16	6955	540	12.88	4894	361	13.55	{2342*	190*	12.33*
							{2061	179	11.51
17	7200	558	12.91	4986	371	13.44	{2394*	188*	12.74*
							{2214	187	11.84
18	7343	567	12.95	5019	369	13.60	{2418*	190*	12.73*
							{2324	198	11.74
19	7737	586	13.20	5244	379	13.84	{2512*	190*	13.23*
							{2493	207	12.04
20	6737	503	13.39	4919	376	13.11	{2088*	159*	13.13*
							{1818	127	14.31
21	6912	508	13.60	4600	343	13.62	{2550*	197*	12.94*
							{2237	165	13.49
22	6887	522	13.19	4762	355	13.41	{2187*	155*	14.11*
							{2125	167	12.72
23	6600	506	13.04	4675	341	13.71	{2237*	–	
							{1925	165	13.56
24	5450	417	13.07	4325	318	13.60	1125	99	11.36
25	6575	500	13.15	4362	327	13.34	{2137*	166*	12.87*
							{2213	173	12.80
	Average		13.25	Average		13.43	Average (11)		13.27*
							Average (25)		12.76
							Grand average		12.93

*By determination. All other globulins by difference.

both by difference and by direct determination. The factor by difference was derived from the ratio between the total protein minus the albumin protein and the total tyrosine minus the albumin tyrosine. There is greater individual variation in the factors obtained for globulin than for those determined for either total or albumin protein. This is true whether the figure was derived by difference or by direct determination. When determined directly there is

TABLE IV

COMPARISON OF RESULTS OBTAINED BY COLORIMETRIC METHOD X AVERAGE FACTOR AND BY MICRO-KJELDAHL METHOD. RESULTS GIVEN IN PERCENTAGE OF PROTEIN

NO.	MICRO-KJELDAHL METHOD			COLORIMETRIC METHOD		
	TOTAL	ALBUMIN	GLOBULIN	TOTAL	ALBUMIN	GLOBULIN
1	6.36	4.21	2.15	6.42	4.53	1.89
2	6.56	4.24	2.26	7.02	4.37	2.65
3	7.23	4.57	2.66	7.32	4.67	2.65
4	7.15	4.79	2.36	7.10	4.78	2.32
5	5.19	2.53	2.66	5.60	2.58	3.02
6	7.11	5.01	2.10	7.06	5.31	1.75
7	7.30	4.63	2.67	7.16	4.64	2.52
8	7.43	4.60	2.83	7.52	4.70	2.82
9	6.91	4.71	2.20	6.98	4.75	2.23
10	6.22	3.89	2.33	6.42	4.07	2.35
11	7.53	4.35	3.18	7.80	4.38	3.42
12	6.63	4.51	2.12	6.62	4.45	2.17
13	6.90	3.69	3.21	7.07	3.72	3.35
14	6.12	4.53	1.59	6.20	4.43	1.79
15	6.56	4.60	1.96	6.68	4.75	1.93
16	6.56	3.88	2.74	6.62	3.96	2.66
17	6.64	4.59	2.05	6.69	4.60	2.09
18	6.52	4.44	2.08	6.86	4.48	2.38
19	6.80	4.73	2.07	6.90	4.70	2.20
20	7.04	5.06	1.98	6.96	5.10	1.86
21	7.10	4.69	2.41	7.54	4.93	2.61
22	6.20	3.00	3.20	6.31	3.18	3.13
23	7.12	4.71	2.41	7.16	4.84	2.32
24	7.09	4.76	2.33	7.40	4.98	2.42
25	7.40	4.69	2.71	7.52	4.96	2.56
26	7.71	5.24	2.47	7.77	5.10	2.67
27	6.77	4.78	1.99	6.67	5.05	1.62
28	6.91	4.60	2.31	6.74	4.61	2.13
29	6.89	4.60	2.29	6.92	4.76	2.16
30	6.67	4.62	2.05	6.70	4.58	2.12
31	5.57	4.34	1.24	5.52	4.27	1.25
32	6.83	4.57	2.26	6.62	4.40	2.22
33	5.70	3.79	1.91	5.79	3.80	1.99
34	6.82	4.61	2.21	7.06	4.65	2.41
35	7.40	4.98	2.43	7.62	4.98	2.64
36	6.48	4.29	2.19	6.83	4.12	2.71
37	7.10	4.70	2.40	7.46	4.64	2.82
38	5.82	4.30	1.52	5.61	4.43	1.18
39	8.27	4.37	3.90	8.54	4.49	4.05
40	6.88	4.97	1.91	7.01	4.66	2.35

much greater chance for error because of the technical difficulty involved in the separation, filtration, washing, and solution of the globulin fraction than is met in either of the other two types of protein. When calculated by difference the variations in the total and albumin factors may be such as to combine to make a wider variation in the globulin factor. Furthermore, the so-called globulin fraction of serum protein is known to be made up of a varying mixture of proteins which may not all have the same chromogenic capacity. At

any rate, although it is of interest to know what the factor for globulin is, the figure is of little use since in practice the globulin in serum is almost universally calculated by subtracting the percentage of albumin from the percentage of total protein. As shown in Table III the average protein equivalents of 1 mg. of tyrosine are as follows:

Total protein = 13.25 mg. Albumin = 13.45 mg. Globulin = 12.93 mg.

Having derived these factors the next step in our investigations has been to test their reliability as compared with the Kjeldahl method for the determination of serum protein. Parallel determinations have been run on a series of 40 serums by our modified colorimetric technic and the micro-Kjeldahl method using the sulphuric-phosphoric-persulphate digestion mixture described

TABLE V

COMPARISON OF COLORIMETRIC RESULTS X AVERAGE FACTOR WITH MACRO-KJELDAHLS DONE IN MEDICAL LABORATORY. RESULTS ARE GIVEN IN PERCENTAGE OF PROTEIN

SERUM	MACRO-KJELDAHL			COLORIMETRIC X AVERAGE FACTOR		
	TOTAL	ALBUMIN	GLOBULIN	TOTAL	ALBUMIN	GLOBULIN
1	5.87	2.77	3.10	5.95	2.45	3.40
2	6.46	3.76	2.70	6.30	3.57	2.73
3*	1.50	–	–	1.49	–	–
4	6.35	4.54	1.81	–	4.39	–
5	5.79	2.76	3.03	5.58	2.46	3.12
6	6.98	4.14	2.84	6.84	3.97	2.87
7	5.61	3.76	1.85	5.41	3.52	1.89
8*	1.62	0.87	0.75	1.66	0.73	0.93
9	6.04	2.76	3.28	6.30	–	–
10	6.70	3.93	2.77	6.66	–	–
11	5.74	2.23	3.51	5.83	–	–
12	6.85	4.43	2.42	6.82	–	–
13*	3.07	2.07	1.00	3.24	–	–
14†	1.30	0.59	0.71	1.38	–	–
15*	1.34	0.71	0.63	1.34	–	–
16	5.72	2.17	3.55	6.14	2.02	4.12
17	6.83	4.37	2.46	6.35	4.30	2.05
18	6.71	4.66	2.05	6.59	4.30	2.29
19	7.97	4.13	3.84	8.09	3.82	4.17
20	4.07	2.44	1.63	4.09	2.32	1.77

*Ascitic fluid.
†Pleural fluid.

by Van Slyke.[7] These serums were samples sent routinely to this laboratory for serum protein determinations. Some contain normal amounts of protein and others are quite pathologic specimens. The small volume of the samples available from infants and children made it necessary to substitute the micro- for the macro-Kjeldahl method in this series. A large number of parallel determinations has, however, shown good agreement between these two variations of the Kjeldahl method. The data in Table IV show the comparative results obtained by the colorimetric and micro-Kjeldahl methods. The percentages of globulin were determined by difference in both methods. The data presented are entirely unselected and represent all the parallel determinations we have made. In analyzing the agreement between the results obtained by these two methods, it must be borne in mind that both are micro-methods and that the experimental error in each approaches 0.20 per cent protein, hence occa-

sional discrepancies as great as 0.40 per cent of protein are inevitable regardless of the accuracy of the factor used.

As a further test a similar series of comparisons have been made between the results of our colorimetric determination of serum protein and those obtained on the same serums with the macro-Kjeldahl method by workers* in an independent laboratory. These results are presented in Table V and show the same agreement as when both procedures were carried out in this laboratory. Included in this series are a few determinations on ascitic fluid and other transudates. It is interesting to note that the same factors are applicable to the determination of protein in material of this type as in blood serum.

From these data it appears that when tyrosine is determined in various types of serum protein by the procedure which we have described, values are obtained which bear a constant relationship to the amount of protein present. Under these conditions the protein equivalents for 1 mg. of tyrosine are 13.25 mg. for total serum protein and 13.45 mg. for serum albumin. A considerable series of parallel determinations show that the procedure and these factors give values for serum proteins which agree closely with the results obtained by the more laborious Kjeldahl method. Serum globulin is probably best determined by difference.

* * * * *

In a conversation with Dr. David Greenberg at the time this work was nearing completion we learned that he had encountered the same difficulties with his original technic and was working out a modification similar to the one we have proposed. Later Dr. Greenberg kindly sent us his manuscript (this JOURNAL **21**: 431, 1936) and also read a preliminary report of our work.

It will be noted that the protein equivalents of 1 mg. of tyrosine determined by Dr. Greenberg are lower than those which we have found. A careful comparison of the procedures described in the two papers shows that Dr. Greenberg heats his total protein and albumin fraction with a greater *concentration* of sodium hydroxide than we have used. The procedure for globulin appears to be the same as ours. A few experiments which we have carried out since the receipt of Dr. Greenberg's manuscript indicate that a somewhat greater chromogenic value is obtained by increasing the concentration of alkali. This would probably account for the lower factors at least in the case of total protein and albumin. If practical experience with the new procedure and factors presented by Dr. Greenberg show equally satisfactory agreement with results by the Kjeldahl method as we have obtained with the method which we describe there seems to be no particular advantage in one procedure over the other. The point must be stressed, however, that whatever method and factors are adopted for the colorimetric determination of serum proteins the prescribed experimental conditions must be closely followed.

*We wish to express our thanks to Mr. J. H. Akeroyd, Jr., of the Department of Medicine for allowing us to present the results of his Kjeldahl determinations and for furnishing the samples of serum for our parallel colorimetric determinations.

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THE INFLUENCE OF THE COMPOSITION OF THE ANTIGEN-EXTRACTS FOR SOME SERODIAGNOSTIC TESTS ON SYPHILIS (M.B.R. II and M.K.R. II)*

BERTIL JOSEPHSON, M.D., DOCENT, STOCKHOLM, SWEDEN

SEVERAL investigators (Georgi, Vermast, Kahn, Meinicke, Laubenheimer, Eagle, Norel, Poeplau, and others) have pointed out that for the flocculation reactions on syphilitic blood there often is a fixed proportion between the concentrations of the suspended substance and the lipoids from the heart extract, at which the reaction turns out most advantageously.

Of the great number of flocculation tests used in the serum diagnosis of syphilis those of Kahn, Müller (M.B.R. II) (see the comparison of Moerch) and Meinicke (M.K.R. II) are commonly used in Sweden. The reagents for the tests of Kahn are well described in several publications. Not very exact accounts, however, are given of the composition of the extracts for the tests of Müller and Meinicke. For this reason I have found it of interest to examine the influence of differences in the composition of the combined lipoid solutions for these reactions.

In both the tests in question the antigenic lipoids are from alcoholic oxheart extracts. The suspended substance is in the M.B.R. II cholesterol and in the M.K.R. II tolu balsam. Eagle has tried to give an explanation of the fact, that the Wassermann and other similar tests become more sensitive with increasing cholesterol content in the antigen solution. Which was now the influence of alterations in the proportion between the concentration of the heart lipoids and the matters to be suspended in the two reactions mentioned above?

THE CONGLOBATION REACTION OF MÜLLER (M.B.R. II)

According to the information given by Müller the reagent used in this test is an alcoholic extract of oxheart (1:30). The extract would have to be concentrated on water-bath and tempered with much cholesterol.

*From the Chemical Department of the Caroline Institute, Stockholm.

Oxhearts were discharged from fat and thoroughly ground. Eighteen hundred grams of the ground hearts were extracted with 15 liters absolute alcohol (Extr. 1), 800 gr. were extracted with 6 liters absolute alcohol (Extr. 2) and 200 gr. also with 6 liters (Extr. 3). Finally 180 gr. were treated with 1.5 liters alcohol (Extr. 4). For all the extractions the shaking machine was used three hours a day during three days. Extr. 1 was then stored for three months at about 22°-27° C. before filtering, Extrs. 2 and 3 for three weeks at room temperature, Extr. 4 for three weeks at 38° C. They then were filtered. Extr. 3 according to Müller's suggestion was concentrated on a water-bath to a third of the original volume and filtered again. The analyses of the filtered extracts gave the following values (Table I). N-analyses Micro-Kjeldahl, P-analyses Pregl.

TABLE I

EXTR. NO.	SPEC. GRAVITY 20° C.	CHOLESTEROL MG./ML.	DRY RESIDUE MG./ML.	ASH MG./ML.	N MG./ML.	P MG./ML.
1	8.33	0.15	6.75	0.67	0.88	0.155
2	8.30	0.13	4.94	0.66	0.25	0.147
3	8.42	0.19	6.43	0.77	0.27	0.169
4	8.36	0.19	5.54	0.54	0.25	0.111

By warming in 38° C. thermostat 0.55 per cent cholesterol was solved in parts of all the extracts. Each extract was then divided in parts and tempered with 3, 3.5, 4, etc. until 7 per cent ether per volume, respectively. After inoculation of some cholesterol crystals the solutions were left standing at 20° C. for two days. As shown by Vermast, the solubility of cholesterol in alcohol is very much dependent not only on the eventual presence of small amounts of water but also on the temperature. The solutions were filtered from crystallized cholesterol and the remaining cholesterol content again determined.

Each filtrate was then tried in the conglobation reaction (the modification with 0.10 and 0.15 ml., respectively) after the instructions given by Müller. A considerable number of Wassermann reaction positive and negative serums were used.

All the experiments with Extr. 3 (prepared after Müller) gave bad results. The precipitates with the positive serums were not clot-shaped and sunk to the bottom of the tubes. Therefore this extract was excluded. Furthermore, all the other extracts with more than 5 per cent ether tended to separate at the dilution with the salt solutions; and in the tubes with negative serums there appeared precipitates on the bottoms of the tubes or small clots ("snowing"). Generally the strongly positive serums with these extracts gave beautiful clots, which now and then had sunk to the bottom. The reaction was oversensitive and the cholesterol content too high.

The extracts with less than 3 per cent ether, on the other hand, did not divide at the dilution. With all the negative serums they gave clot-free suspensions, which, however, often kept a little smeary precipitation. With some of the positive serums they showed no clotting, only a flocculation. The reaction was not very sensitive and the cholesterol content was too low.

Very good reactions with both the negative and the positive serums were obtained by using Extrs. 1 and 4 with 4 per cent ether, and Extr. 2 with 3 per cent ether. In these samples there was found for Extr. 1, 0.53 per cent cholesterol, in Extr. 2, 0.39 per cent, and in Extr. 4, 0.45 per cent. These fractions

gave very beautiful reactions, especially in the case of Extr. 1, but also of Extr. 4. The clots observed in syphilitic serums with Extr. 2 were sometimes a little thin and ramified.

Now big quantities of the three solutions just mentioned were prepared and tried on usual serums and cerebrospinal fluids, sent in to the serologic laboratories for testing for syphilis.

Extracts, made as number 1, have been used in about 20,000 cases, of which about 1,000 were positive. In all cases the Wassermann reaction also was made in various forms, and in many of them Kahn's standard test and Meinicke's M.K.R. II as well. On about 3,000 of these serums the clotting test of Müller also has been carried out at the same time with the original German "Ballungsreagens." In all cases the results with my extract have shown an extremely good correspondence with the other reactions and with the informations from the clinics. In the same way and with nearly similar results Extr. 2 has been tested on about 500 samples and Extr. 4 on about 1,200.

Thus the best way to prepare this reagent seemed to be the one used for Extr. 1 or possibly Extr. 4 with 3 or 4 per cent ether.*

The reactions seemed to be more clear and selective when the cholesterinized extracts had been left to ripen for three to five months.

It must be mentioned that Poeplau has made an investigation of the cholesterol content suitable for this reaction and found it very definite. He also preferred the nonconcentrated extracts. However, he seems to have tried only one single heart extract, and he does not mention any analyses. He concluded that the reagents were oversensitive when there was too little cholesterol.

THE CLARIFICATION TEST OF MEINICKE (M.K.R. II)

Meinicke states that the reagent for his M.K.R. II consists of an alcoholic extract of ground oxheart, which has been dried at 56° C., extracted with ether and dried again. The reagent should also contain 1.4 per cent tolu balsam and 0.01 per cent victoria blue. The latter figures are also found in works by Boas and Izikowitz. I have not been able to find any exact description for the preparation of the heart extract for this, the latest reaction of Meinicke.

Oxhearts were well ground, dried at 56° to 58° C. on glass plates and ground again. Three hundred grams of the powder were extracted for five days with ether, the first day by aid of machine shaking. Ten milliliters ether were used for each gram of the powder. The ether was filtered off and the residue again extracted with the same amount of ether. After drying at 37° C. one-third of the powder was extracted with 0.9 liters of alcohol 95 per cent (Extr. A), one-third with 2 liters (Extr. B) and one-third with 0.5 liters (Extr. C), respectively. Also this extraction continued for five days, the first one in the shaking machine. The analyses are shown in Table II.

TABLE II

EXTR.	DRY RESIDUE MG./ML.	ASH MG./ML.	N MG./ML.	P MG./ML.
A	9.59	1.025	0.75	0.238
B	5.02	0.518	0.42	0.121
C	15.68	1.619	1.04	0.419

*Extract of the Type 1 is now kept for sale by The Astra Lmtd, Södertelje, Sweden.

Now dilutions with alcohol were made of these extracts containing 5 per cent, 10 per cent, 20 per cent and so on, of the extract. Each of these solutions, again, was divided in parts, in which was dissolved 1.0 per cent, 1.1 per cent, 1.2 per cent, etc., of tolu balsam. To all of them there finally was added 0.002 per cent victoria blue (Herzberg-Hollborn).

These solutions were tested in the "Klärungsreaktion" on positive and negative serums. The instructions given by Meinicke were scrupulously followed. Between those samples, which seemed to give the best results, closer dilutions were made. It turned out that as already pointed out by Meinicke the best reactions were obtained by using a definite proportion between the tolu balsam content and the content of solids from the original extract. With this proportion the different extracts showed similar activity. At low concentrations, 1.1 to 1.6 per cent balsam, this proportion was about 13, but at increasing concentration (2 to 3 per cent) it was somewhat lower, about 8 to 10. If there was too little balsam, the negative tests remained homogene, but the positives showed a turbidity, increasing with decreasing balsam concentration until the reaction seemed negative. The extract was not sensitive enough. A decrease of the heart extract concentration, again, caused an increasing clarification of the negative samples, the positive remaining manifest. It was too sensitive. The allowed limits for the proportion seemed to be much more narrow at low concentrations than at high.

Altogether the reaction was more distinct at rather low concentrations, the best reagents containing 1.2 to 1.3 per cent balsam and 0.09 to 0.10 per cent heart solids. The test then turned out very clear. The results with the different extracts were closely corresponding.

The concentration of victoria blue may be varied within wide limits. Using the Herzberg preparation from Hollborn, which proved to be the best of several tested, the ideal concentration was 0.0015 to 0.0020 per cent. (Meinicke directs 0.01 per cent.)

Five such extracts of Type A* were prepared from different hearts and their reacting compared with the other syphilis tests as for the conglobation reagent. These extracts all reacted similarly. Comparison was also made with the original German M.K.R. II extract. Until now about 10,000 cases (about 200 cerebrospinal fluids) of which about 900 positives have been examined with the new extracts, have all given results corresponding well with those, found with the other reactions, and also with the reaction carried out with the original M.K.R. II reagent from the "Adler-Apothek" in Germany. In all cases the macromodification of Meinicke was used. In some cases also the micromodification was tested.

SUMMARY

An investigation of some different reagents for the conglobation reaction of Müller (M.B.R. II) and the clarification reaction of Meinicke (M.K.R. II) is described. It is shown, that for these reactions the proportion between the oxheart lipoids and the substance, to form the suspension (cholesterol, tolu balsam) must be of a definite magnitude. Too much heart lipoids will make the reaction less sensitive and too little the reverse. Confirming the theory of

*This extract now is kept for sale by The Astra Lmtd, Södertelje, Sweden.

Eagle, this may be due to a lability in the colloidal antigenic suspension. The less of the charging heart lipoids, the easier the suspension will be discharged and precipitated. The proportion ought to be tested out for each heart extract. The conglobation reagent for the Müller test ought to contain 3 or 4 per cent ether to keep sufficient cholesterol (0.50 to 0.55 per cent) in solution. For the M.K.R. II the proportion between tolu balsam and heart lipoids should be about 13 and the total concentration 1.4 per cent. The convenient concentration of the suspension system must within certain limits be determined and fixed.

It is a pleasure to me to express my thanks to Docent H. Davide for his kindness in helping me to try my extracts in his serologic laboratory. I also wish to thank Miss Dahlqvist, who has assisted me in the testing of the extracts.

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A NEW DILUTING PIPETTE FOR THE SAHLI HEMOGLOBINOMETER*

Helmuth Ulrich, M.D., Boston, Mass.

THE pipette to be described is designed to be used with the Sahli hemoglobinometer. It has two distinct advantages: (1) It obviates the necessity of carrying the entire Sahli apparatus to the patient's bedside or home; (2) it permits making the estimation of hemoglobin at a convenient time subsequent to obtaining the blood.

In principle, it is similar to the diluting pipettes that are used for the estimation of the number of erythrocytes and leucocytes. It differs from the usual Sahli pipette only in having a bulbous dilatation above the 20 c.mm. mark. The capacity of the bulb is approximately equal to that of the Sahli tube to the mark 10. The 20 c.mm. mark is its only graduation. The initial dilution of the blood with hydrochloric acid is made in the pipette rather than in the Sahli tube.

Directions for Use.—Draw the blood from the usual puncture wound of the ear or finger (or oxalated blood) exactly to the 20 c.mm. mark; then

*From the Evans Memorial of the Massachusetts Memorial Hospitals and Boston University School of Medicine.
Received for publication, July 16, 1935.

aspirate sufficient tenth normal hydrochloric acid to fill the bulb. If the estimation is not to be made at once, close the openings of the pipette with a wide rubber band. When ready to proceed, eject the contents of the pipette into the Sahli tube and add water in the usual manner until the color of the diluted blood matches that of the standard.*

It is probably correct to state that many estimations of hemoglobin by the Sahli method are made without allowing sufficient time for the full development of the color of acid hematin. The usual directions that accompany Sahli outfits or are given in textbooks (if they are stated at all) specify that a few minutes should elapse before the comparisons are made. Nicholson[1] claimed that "95 per cent of the acid-hematin color" will develop in ten minutes, but according to Osgood[2] "the reaction . . . . . requires at least twenty-four hours at room temperature to become complete." They recommended

Fig. 1.

immersion of the tube containing the blood and acid mixture in a beaker of warm water (55°-60° C.), at which temperature "the reaction is completed in seven minutes." For practical purposes, one hour at room temperature appears to be sufficient, as is shown by the results obtained by the Sahli method in four cases (see Table I).

TABLE I

SHOWING THE EFFECT OF TIME ON THE COLOR OF ACID HEMATIN

CASE	READING					
	IMMEDIATE	1 HOUR	2 HOURS	6 HOURS	24 HOURS	48 HOURS
1	67%	72%	70%	71%	71%	70%
2	79%	83%	81%	84%	82%	
3	91%	100%	100%	101%	100%	100%
4	89%	93%	94%	92%	94%	

The minor variations recorded after the first hour cannot be ascribed to changes in the color, but must be accepted as errors inherent in colorimetric determinations. The essential but frequently neglected time interval is an additional point in favor of using the special diluting pipette.

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2. Osgood, E. E.: Laboratory Diagnosis, Philadelphia, 1935, P. Blakiston's Son & Co., p. 396.

99 BAY STATE ROAD

*The pipette and a hemacytometer case with an extra groove for it may be purchased on special order from Arthur H. Thomas Co., Philadelphia, Pa. Both will be established as catalogue and stock items, if a sufficient demand develops. The additional groove in the hemacytometer case may be used also for a spare red or white pipette and should prove convenient for those who use a white pipette for hemoglobin determinations by the Haden-Hausser method.

A SYNCHRONOUS MOTOR ELECTRIC TIME CLOCK*

A. L. Bennett, Ph.D., Omaha, Neb.

THIS clock provides electrical contact at intervals of 1, 5, 15, 30, and 60 seconds as well as 5, 15, 30, and 60 minutes without adjustment of any clock contacts. Two switches provide simple and instantaneous setting for any of the intervals. Contact is short and uniform regardless of interval. The clock is accurate, dependable, and entirely adequate for timing research experiments or

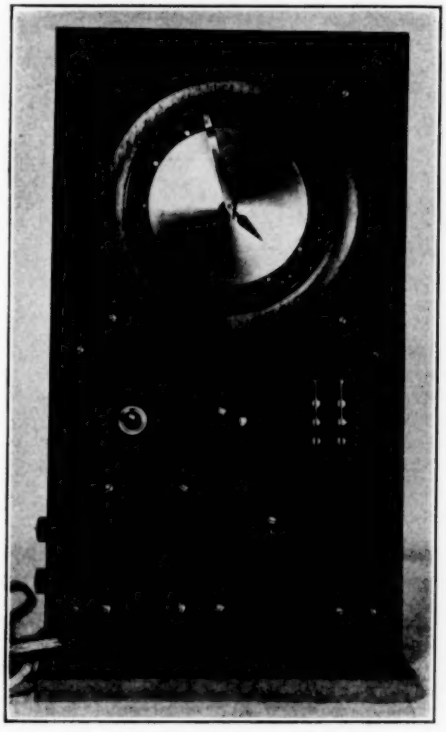

Fig. 1.

as a master clock for a general laboratory. Furthermore it may be easily made in a laboratory workshop at a very nominal cost.

A small synchronous electric clock motor is mounted with the central pinion extending through a panel of insulation material in such a way as to permit replacement of the hands and use of the panel as a dial for the clock. Contacts are inserted through the panel so that the "second" hand makes contact with the outer circle of sixty points while the "minute" hand reaches the inner circle of twelve points. All wiring is done on the back of the panel. The front

*From the Laboratory of Physiology and Pharmacology, University of Nebraska Medical School.

Received for publication, June 6, 1935.

of the dial is polished so that the contacts are flush with the surface and the hands slide smoothly over metal and insulation material alike. Dimensions may be made to suit the particular clock motor which is used. Following are those used by the author:

Radius for circle of outer contacts	47.0 mm.
Radius for circle of inner contacts	42.0 mm.
Diameter of outer contacts	1.3 mm.
Diameter of inner contacts	2.9 mm.

The outer circle of dial contact points which furnish the "second" intervals from 1 to 60 are made of brass brads which are driven through the panel

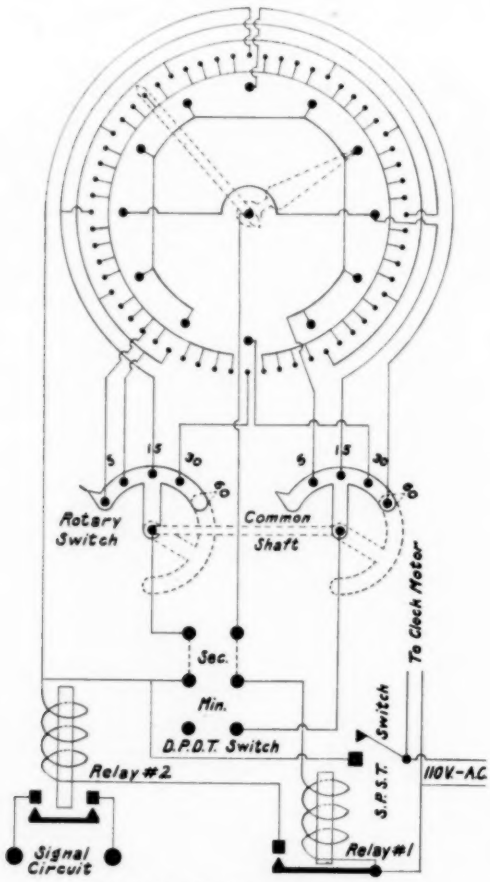

Fig. 2.—Diagram of circuit.

after it is drilled with holes that provide a very snug fit. The larger contacts of the inner circle, which provide the "minute" intervals from 5 to 60, are made of brass wood screws. These are turned into snugly fitting drill holes until the smooth section of the screw has entered the panel. The head is clipped off and then screws and brads alike are filed flush with the dial surface, leaving each projecting on the back of the panel for solder connections. The front of the dial must be polished carefully so that the hand contacts will pass over it

smoothly. This is best done after the soldering has been completed on the back of the dial, as otherwise the heat may roughen the surface already polished.

The hand contacts are made by cutting narrow strips from the two halves of a gold fountain pen point and soldering these to the "second" and "minute" hands of the clock. These points are adjusted so that the iridium tips contact the brass points of the dial. The "second" hand moves more smoothly if the tip is made to follow the hand by about one-fourth of an inch and is thus "dragged" over the dial surface. If the second hand is too flexible, it may be replaced with a strip of "tin."

The main control switch is a double bank, four contact, Yaxley radio switch. This is made accumulative rather than selective by replacing the single rotating contacts with strips of metal which will cover all four of the stationary contacts when the switch is turned to the "stop" toward the left. This provides in one control the selection of interval. The small double-pole, double-throw radio switch determines whether the interval shall be in seconds or minutes. A standard radio toggle switch is used to close the relay circuit. Reference to the wiring diagram will make this clear.

In order to use the 110 V-A.C. current to activate the relays as well as run the clock, a 10,000 ohm, sensitive relay is placed in circuit with the clock contacts. This draws only 2.5 milliamperes and thus protects the clock contacts from burning and becoming rough. A second relay, activated through the first, has heavy enough contacts to carry the load of several signals if the clock is to be used as a master timer for a large laboratory.

Specifications for the materials used by the author:

Clock, General Electric No. AB3F62
Rotary Switch, Yaxley No. 1326
Relays, Struthers Dunn Inc., Philadelphia
No. 1, Type CXB51, 10,000 ohm, A.C. coil.
No. 2, Midget, Type ABTX$_1$, A.C. coil.
Panel, Insuline, $7 \times 12 \times 3/16$ inches

If the above clock is used, the sides and back of the case may be fastened to the back of the panel to provide protection for the motor and electric connections. The relays and switches may then be covered with a separate case. Since there is no adjustment necessary at the clock contacts a glass cover may be fastened tightly over the dial, thus making it dust proof.

THE IMPORTANCE OF LEUCOCYTE COUNTS IN PHAGOCYTIC TESTS*

RUTH WESTLUND JUNG, B.S., M.S., PH.D., CHICAGO, ILL.

IN DETERMINING the opsonic index of an individual's serum by Wright's method the number of leucocytes is not important since the same leucocyte suspension is used for both the test and control serums. When, however, one compares the phagocytosis occurring in one sample of whole blood with that present in another the number of leucocytes should be taken into account. The importance of knowing the number of leucocytes used in phagocytic tests was suggested more than thirty years ago, yet some present-day investigators disregard this point in describing their "quantitative" tests for the phagocytic power of an individual's blood.

Dodd[1] in 1907 called attention to the discrepancy in Leishman's method of obtaining opsonic indices by comparing the amount of phagocytosis in the whole blood of an individual with that occurring in the blood of another person. He realized that the differing number of leucocytes in the blood was the cause and he attempted to remedy it by applying a simple formula as follows. Counts were made on the mixtures of bacteria and blood used in the test, employing a red blood cell counting pipette. To correct the apparent index he multiplied the control index, obtained by Leishman's method, by its corresponding number of leucocytes and divided the result obtained into the other index multiplied by its corresponding number of leucocytes. In this way he secured results approximating those obtained by Wright's method. Below is shown an example to illustrate the method.

	NO. BACTERIA PER 100 CELLS	LEISHMAN INDEX	WRIGHT INDEX	NUMBER OF LEUCOCYTES
Control A	190	1.0	1.0	11000
B	115	0.6	0.9	16000
	$\frac{0.6 \times 16000}{1.0 \times 11000} = 0.87 =$ corrected index			

Fleming[2] in 1908 observed higher readings with fewer corpuscles. A fivefold dilution of the leucocytes caused a 50 per cent increase in the phagocytic index. Twofold dilution gave results 10 to 26 per cent higher than when undiluted blood was used. He considered the bacterial emulsion of the greatest consequence to accuracy and the leucocyte suspension next. Doubling the volume of leucocytes reduced the index to 0.8 while using one-half the volume increased it to 1.15. Doubling the volume of bacterial suspension increased the index to 1.33. Using one-half the volume decreased it to 0.68.

*From the Department of Bacteriology, Northwestern University Medical School.
Received for publication, June 28, 1935.

Hektoen[3] in 1911 wrote that one can compare the phagocytic properties of leucocytes from a particular source with those of other leucocytes by finding the amount of phagocytosis by each only under strictly comparable conditions. For such purposes the phagocytic mixture which is the same in everything except the source of leucocytes self-evidently must contain the same number of leucocytes. He suggested that a leucocyte count be made and that the suspensions be made comparable by dilution with NaCl. He adds that it is quite evident in mixtures containing an unequal number of leucocytes there will be less phagocytosis per phagocyte in a mixture containing larger numbers of leucocytes and vice versa. In other words an apparent difference in phagocytic power may result from an inequality in the number of leucocytes in the mixture studied. He cites the experiments of several investigators and notes their failure to take the number of leucocytes into consideration.

EXPERIMENTAL

The recognition of the importance of the leucocyte count, in the phagocytic test, by some of the earlier workers and yet completely ignored by some present-day investigators led us to investigate this question further.

Since the present tendency seems to be to evaluate phagocytosis by the percentage of leucocytes containing bacteria or particles rather than the actual number of bacteria or particles contained in the cell, the findings reported in this article are on that basis. The present paper is an expansion and continuation of an earlier report.[4]

The question arose of how to obtain samples of blood alike in all respects except the number of leucocytes contained. Since no attempt was made to separate the leucocytes from the red blood cells, diluting with salt solution, which would reduce the number of leucocytes, would also change the number of red blood cells and the conditions in two samples of blood would not be comparable. A few experiments were conducted in this way, however, merely to give an indication of the relation of the number of phagocytes to the percentage phagocytosis. It can readily be seen from Table I that as the blood is diluted the percentage of phagocytosis increases.

TABLE I

DILUTION OF BLOOD	PERCENTAGE OF PHAGOCYTOSIS			
	EXPER. 1	EXPER. 2	EXPER. 3	EXPER. 4
Undiluted	32	43	40	36
1:2	43	48	47	41
1:3		58	71	53
1:4	53		72	74

To determine that the number of leucocytes was the responsible variable all other inconstants were removed by using deleucocyted blood as a diluting fluid, thus permitting the numbers of leucocytes to be varied without changing the red blood cell count. The blood was deleucocyted by a modification of the method of Fleming.[5] For counting the leucocytes it was necessary to find another counting solution since the usual 1 per cent acetic acid did not completely dissolve the heparinized or citrated washed erythrocytes. These appeared in large clumps and interfered seriously with the leucocyte count. Experiments

designed to obviate this difficulty indicated that the use of heparin or citrate was not responsible but that centrifuging and washing with salt or Tyrode's solution caused this phenomenon. The addition of 0.8 per cent $CaCl_2$ to the 1 per

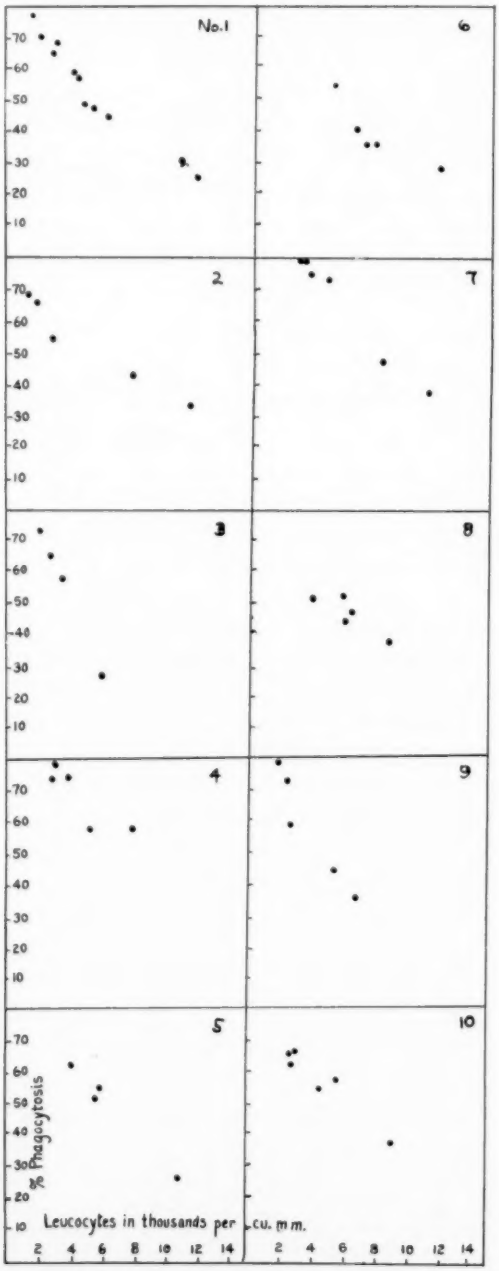

Chart 1.—Correlation between leucocyte count and percentage of phagocytosis in ten normal human male adults.

cent acetic acid solution lessened the difficulty to a great extent and was used in the earlier experiments. A 0.5 per cent HCl solution was subsequently found

more satisfactory and was employed in the later work. It is essential, however, that the leucocytes be counted as soon as mixed with the counting fluid since the HCl has a tendency to dissolve the leucocytes if left standing for any length of time. Counts of leucocytes on uncentrifuged blood with 1 per cent acetic acid and 0.5 per cent HCl were comparable.

After preparing samples of blood containing varying numbers of leucocytes phagocytic tests were run using a heat-killed culture of streptococcus and serum from the blood of the individual tested. The usual capillary tube method was employed. The results showed that this method was as accurate for this purpose as the more recently adopted methods of keeping the materials in agitation during incubation of the test. Fifteen minutes at 37° C. was chosen as the incubation time. While the leucocyte count made on the heparinized blood is not an exact evaluation of the number used in the test it gives the relative numbers in each sample of blood, and this is the important point.

On plotting leucocyte concentrations as abscissae and percentage phagocytosis as ordinates it was clear in every instance that the two quantities were

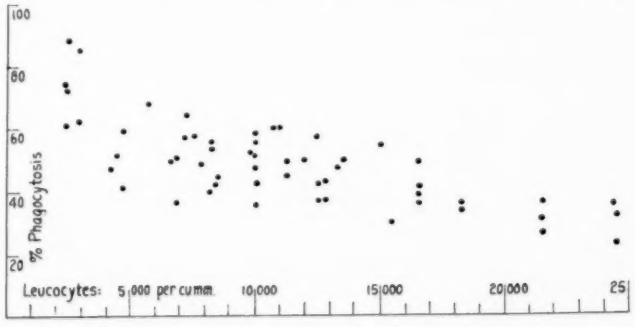

Chart 2.—Correlation between leucocyte count and percentage of phagocytosis in ten normal dogs.

related. Fifty-six determinations were made on 10 normal male adults and 56 on 10 normal dogs. The relation of percentage phagocytosis to the number of leucocytes appeared to be linear so that for a given subject the data could be represented by a line whose slope was equal to $\frac{y_2-y_1}{x_2-x_1}$ where x = the number of leucocytes and y the percentage of phagocytosis. The slope is negative. Its mean value for the ten human males tested was –0.006. The linearity did not extend, however, beyond the limits of 1,500 on the one hand and 12,000 on the other, and while it was believed that the slopes calculated above were measures of the phagocytic power of the individual's blood, nevertheless it proved more convenient to judge such power by direct inspection of the plotted data rather than from calculation. Since the range of leucocytes is greater where dog's blood was used it becomes clear that the curve is not a straight line.

The results with the normal human blood are shown in Chart 1. Each individual case has been considered separately. The effect of changing the number of leucocytes is evident. The results from a study of the blood of the ten dogs have been put together in Chart 2. One notes here the straightening

out of the curve as the higher numbers of leucocytes are encountered. It also shows the general agreement between samples of blood from different dogs.

The following experiments were planned to meet the criticism of Mudd[6] that the results obtained in an earlier report were due to the technic practiced. The suggestion was made that agitation and the employment of an "excess" of bacteria would obviate the "error." Just what is meant by "excess" is not clear. A suspension containing 800,000,000 living staphylococci per c.c. was used as suggested by Mudd and the same streptococcus suspension employed in earlier experiments. This was a heat-killed culture of a hemolytic streptococcus in saline suspension of about the same density as the staphylococcus suspension.

In the first experiment 0.3 c.c. of whole heparinized dog's blood was added to each of a series of tubes. Each sample of blood contained different numbers of leucocytes. Otherwise they were the same. To each tube was then added 0.1 c.c. of the streptococcus suspension. To a second series of tubes of the same blood was added a more concentrated suspension of the streptococcus. This was accomplished by centrifuging the original suspension and resuspending the sedimented bacteria in about half the amount of saline originally used. The tubes were then placed in a small rack and oscillated by hand at room temperature for ten minutes. Four rotations through an angle of approximately 160° were made per minute. The tubes were then placed in ice water, smears made and stained by Wright's method. One hundred polymorphonuclear leucocytes were counted in each smear.

EXPERIMENT I

NUMBER OF LEUCOCYTES*	PER CENT PHAGOCYTOSIS	
	DILUTE SUSP.	HEAVY SUSP.
8100	36	98
8100	44	100
5100	58	99
4300	51	100
3300	69	99

*Since whole heparinized blood was used the leucocyte counts could be made with one per cent acetic acid.

The test using a dilute suspension shows the effect of varying the number of leucocytes. With the concentrated suspension this effect is not apparent. This also shows the importance of using a properly prepared suspension.

In Experiment II one series of paraffin stoppered tubes containing 1 c.c. of heparinized whole dog's blood and 0.3 c.c. of a suspension of living *Staph. albus* (800,000,000 per c.c.) and a second series of tubes containing 1 c.c. of the blood and 0.3 c.c. of the killed streptococcus suspension were placed in a rotating machine making 16 rotations per minute. The machine was set in a 37° C.

EXPERIMENT II

NUMBER OF LEUCOCYTES	PER CENT PHAGOCYTOSIS	
	S. ALBUS	ST. HEMOLYTICUS
13200	89	53
10600	100	78
6100	99	99
4300	97	100

incubator and run for about ten minutes. The tubes were then placed in ice water, and smears made in the usual manner.

It is quite obvious that 0.3 c.c. of the staphylococcus suspension was too much. The results with the streptococcus suspension demonstrate the influence of the number of leucocytes.

Experiment III was planned to remedy the error due to the use of too many bacteria. Consequently 0.1 c.c. of the bacterial suspension was used with 1 c.c. of blood. The tubes were rotated at 37° C. as in Experiment II except that smears were made both after five minutes and ten minutes incubation, the tubes being placed in ice water while smears were being made.

EXPERIMENT III

	NUMBER OF LEUCOCYTES	AVERAGE NUMBER OF S. ALBUS PER PHAGOCYTE	PER CENT PHAGOCYTOSIS S. ALBUS	PER CENT PHAGOCYTOSIS ST. HEMOLYTICUS
After 5 min. inc.	7200	2.4	46	29
	5500	1.5	45	43
	4100	2.4	49	66
	2800	2.4	65	63
After 10 min. inc.	7200	1.9	47	22
	5500	2.9	70	57
	4100	2.5	65	73
	2800	3.3	78	79

Although the variation in the number of leucocytes is not very great, a tendency toward higher percentages with fewer leucocytes is quite plain. This is somewhat more apparent after ten minutes' incubation than after five minutes'. Even the phagocytic index for *S. albus* is seen to increase as the number of leucocytes decreases.

It is evident from these findings that the number of leucocytes present in the phagocytic test is a significant factor even when "agitation" is used.

SUMMARY

The results of the experiments here reported show clearly the importance of a knowledge of the number of leucocytes engaged in phagocytic tests especially if samples of blood from different sources or from the same individual at different times are to be compared.

The relation of the leucocyte count to the percentage phagocytosis is brought out. By applying a simple formula one can compare the phagocytic power of an individual's blood at different times. When enough points are available the graphic method can be substituted for the mathematical.

CONCLUSIONS

Comparisons of percentage phagocytosis based on phagocytic mixtures containing unequal numbers of leucocytes are unreliable. A method for estimating the phagocytic power of the blood, which takes this into consideration, is presented.

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A ROUND TABLE FOR USE IN PATHOLOGIC HISTOLOGY*

J. McLean, M.D., New York, N. Y.

THIS table was designed to seat and provide comfortable working conditions for eight persons engaged in the examination of sections (Fig. 1). It was made circular in shape to secure freedom from elbow touching and an uninterrupted stream of light from a central source as illustrated. The supporting structure is hexagonal in form and contains twelve cabinets, necessarily somewhat tri-

Fig. 1.—Eight persons can comfortably use the table. There is room for microscopes, trays of slides, reference books, etc.

angular in shape, which easily accommodate the largest microscope box, as well as a book or two and some accessories. The main top of the table is for the unobstructed use of the microscope, the writing of diagnoses and perhaps the consultation of a reference book. The trays containing the slides being examined

*From the Memorial Hospital.
Received for publication, June 9, 1935.

are on the smaller table top. These can be reached by all, or passed from one to another. The sides of this smaller table top provide recesses for reprints, ash receivers, etc.; as well as compartments of shelves for the storage of seven trays comparatively free from dust (Fig. 2).

The circular form of the table economizes space; but what is more important, it makes discussion easier and more direct. The round table is adapted to seminars in pathology, biology, or other branches of science employing the microscope.

Fig. 2.—Showing light (suspended by [illegible] chain), cabinet and top table detail.

The table was originally designed for the use of the eight Fellows and four internes of this institution, in order to provide reasonably safe storage for their microscopes and convenient facilities for the daily study of the current sections of the Department of Pathology.

However, formal postgraduate instruction in the Memorial Hospital, while confined to the above groups, is actually given informally to others. For instance, pathologists of several hospitals in the Metropolitan area attend the weekly clinical conference of the Medical Staff, at which interesting diagnostic or therapeutic problems or results are presented with the patients; and spend the afternoon in the pathology department studying the slides which have come through during the preceding week. Then, in addition, many pathologists spend

from a few weeks to a few months (especially in the summer months) studying the daily slides made up of biopsies, operative and autopsy material of this hospital, as well as many sections sent here from widespread sources for diagnosis. There is also available the Ewing Tumor Collection of 5,000 slides, easily accessible in a cabinet next to the table; and a Special Collection of sections selected from time to time from the daily material which illustrate certain pathologic conditions, or are sections difficult to classify exactly. Finally, as the

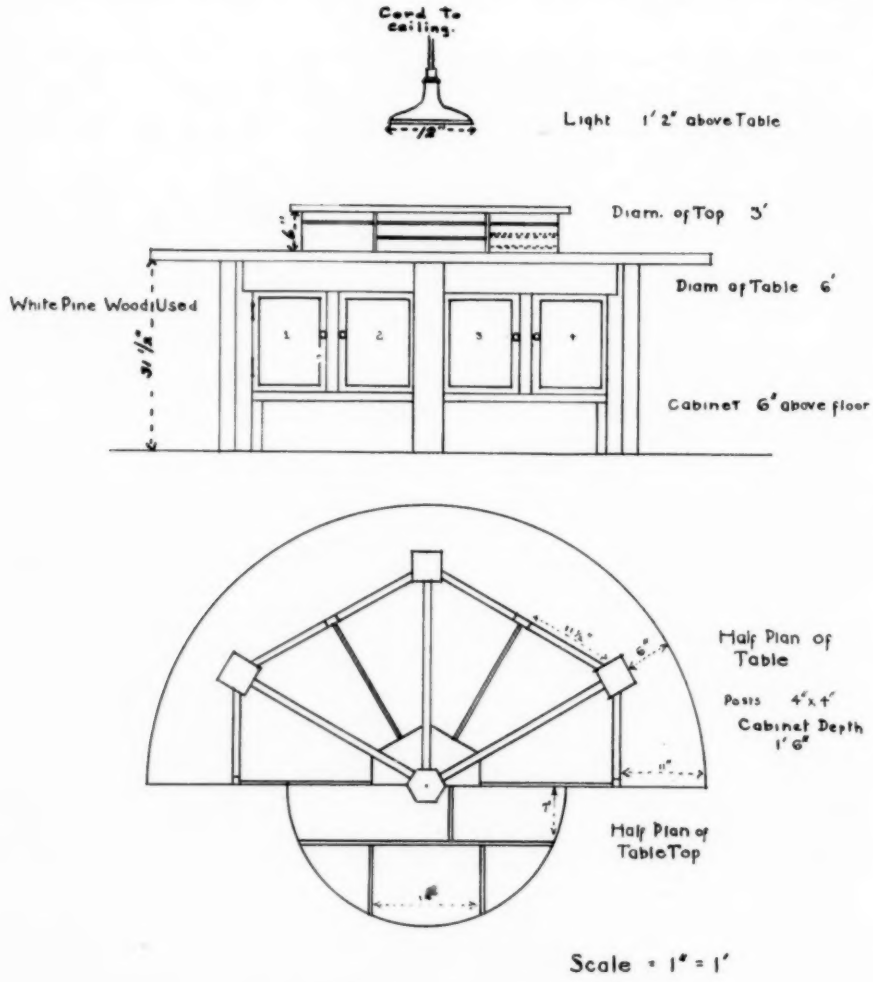
Cord to ceiling
Light 1' 2" above Table
Diam. of Top 3'
Diam of Table 6'
White Pine Wood Used
Cabinet 6" above floor
Half Plan of Table
Posts 4"x4"
Cabinet Depth 1' 6"
Half Plan of Table Top
Scale = 1" = 1'

Fig. 3.—Plan and elevation of table.

round table is located in the Museum, there are 750 gross specimens plainly displayed, and for each there is a large envelope containing the sections thereof and the clinical history of the case, together with photographs and roentgenograms. As the Museum is next to the pathologic laboratory, the gross operative material can be conveniently examined as it comes from the operating rooms.

During the academic year the table is likewise of considerable convenience for under-graduate instruction. The Memorial Hospital offers the senior class

students of the Cornell University Medical College (an affiliate) a four weeks' systematic course in cancer. The students electing the course are received in groups of eight every month of the academic year.

The table has been in use over a year, and its use has not suggested any improvement in design.

Several of the pathologists from other laboratories who have studied here have expressed a desire to have such a table built at their institutions; and for this reason the plan and elevation of the table, photographs, and the following data are given.

The table was constructed in the carpenter shop of this hospital by a master carpenter regularly employed here. Due to the present economic conditions it had to be built as reasonably as possible, yet staunchly to make the expenditure an investment. The main top is of whitewood boards, 12 inches by 1½ inches, and is six feet in diameter. The small top is of five-ply wood and is three feet in diameter. The cabinets were made of white pine and each has a lock. Tops are painted black and waxed; other wood is stained a dark cherry color. The proper elevation of the main top, as well as the height of the lamp, was carefully considered. These are given in the drawing (Fig. 3). The lamp was furnished by the North Light Corporation, 30 West 15th Street, New York City. It contains a 200 watt Mazda lamp, the light of which passes through a blue stained glass cut to diffuse the light. This arrangement and location of the light gives adequate illumination, equally to all working at the table.

Cost of the construction of the table follows:

Lumber	$46.70
Hardware	20.00
Carpenter's wages	65.00
Painter's wages and material	6.50
	$138.20

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

FRUCTOSURIA, Essential, Silver, S., and Reiner, M. Arch. Int. Med. 54: 412, 1934.

Three cases of essential fructosuria are reported, and metabolic studies of two are presented.

This rare anomaly is viewed as a specific, probably inborn, error of metabolism localized primarily in the liver, where the authors believe, a specific enzymatic deficiency exists resulting in impaired ability to fix fructose as glycogen. The metabolism of other carbohydrates is undisturbed. The relative insignificance of insulin and epinephrine in the metabolism of fructose compared with dextrose is pointed out, and the possible intermediary metabolism of sorbitol is discussed.

HEMOGLOBIN, Bile Pigment and Regeneration of the Effect of Bile Pigment in Cases of Chronic Hypochronic Anemia, Patek, A. J., and Minot, G. R. Am. J. M. Sc. 188: 206, 1934.

Nine selected patients with chronic hypochromic anemia were studied to determine whether bile pigment could assist in hemoglobin production.

Concentrated bile pigment alone caused not a reticulocyte response but an increase of hemoglobin, about 7 per cent in ten days. This indicates that in certain anemic patients bile pigment can be absorbed from the gastrointestinal tract for building hemoglobin.

After a reticulocyte response occurred to a suboptimal dose of iron, bile pigment was fed directly with the same dose of iron, and there followed a second reticulocyte response. The second response was sometimes of greater magnitude than the first. This indicates that bile pigment in some unknown manner can facilitate either iron absorption or utilization.

One patient who could not obtain in fourteen months a normal hemoglobin level with large daily doses of iron promptly increased her hemoglobin concentration when bile pigment was fed in addition to iron.

It is suggested that in certain cases of hypochromic anemia there may be a deficiency of a useful material that is contained in bile pigment in addition to iron deficiency.

POLIOMYELITIS, an Enterodepressant Fastor in the Stools and Spinal Cords of Monkeys Infected with, Toomey, J. A., and Von Oettingen, W. F. Am. J. Dis. Child. 48: 30, 1934.

Potent poliomyelitis virus suspensions in physiologic solution of sodium chloride acted as a depressant on the rabbit's intestine. The depression was probably local.

Serum from normal monkeys and from human beings in the acute stages of poliomyelitis did not prevent depression of the intestine, while convalescent human and monkey poliomyelitis serums did.

There was a toxic factor in the stools of monkeys with experimental poliomyelitis which was absent in those specimens obtained from normal monkeys.

None of the specimens of stools obtained from human beings with contagious diseases, except influenza, contained a depressant factor.

LYMPHOGRANULOMA INGUINALE, Cultivation of Virus of, Tamura, J. T. J. A. M. A. **103**: 408, 1934.

The author believes that he has succeeded in cultivating the virus by the following method:

When pus is removed aseptically from a bubo that has not been exposed to external contamination and is found to be bacteriologically sterile, it is diluted 1:5 with sterile saline solution. Now, when from 0.02 to 0.03 c.c. of the diluted pus is planted in the Tyrode's solution containing a piece of guinea pig kidney or liver and incubated at 37.5° C., a peculiar cloudiness appears throughout the supernatant fluid in from thirty-six to forty-eight hours. Control tubes of the medium incubated at the same time remain perfectly clear. This cloudiness can be transmitted from tube to tube and in one instance was carried through twenty-four subcultures, when the procedure was discontinued.

When transfer is made from twelve-day-old cultures it is successful. In a fourteen-day-old culture the cloudiness seems to settle out and when the clear supernatant fluid is subcultured it no longer produces cloudiness.

LEPROSY, Mitsuda's Skin Reaction in, Hayashi, F. Internat. J. Leprosy **1**: 31, 1934.

Preparation of the Vaccine: Fresh nodules are boiled in physiologic salt solution for from thirty to sixty minutes, and ground in a mortar. To 1 gm. of the ground nodular material are added 20 c.c. of the salt solution used in the boiling, fresh salt solution being added, if necessary, to make up the required volume. The whole is filtered through gauze and the filtrate heated at 60° C. for one hour. Carbolic acid is added to make 0.5 per cent concentration. One-tenth cubic centimeter of the material prepared in this manner is used for an intracutaneous injection.

The reaction: The reaction is looked for on the eighth, sixteenth, and twenty-fourth days after the injection, as late reactions may not appear before the second or third week. In the negative reaction in nodular leprosy there is slight reddening which lasts only from two to four days, and never over eight days.

In reading the positive reactions, those with infiltrated areas 0.3 to 0.5 cm. in diameter are classed as one-plus (+), those 0.5 to 1.0 centimeter as two-plus (++), and those over 1.0 cm. and those with pus formation as three-plus (+++). Some reactions start eruptively, while others begin more diffusively, the latter type with large areas gradually becoming strongly positive.

Results obtained: Of the 192 lepers tested, 64 were neuromacular and 125 nodular cases. Of the former all but two (97 per cent) showed positive reactions, while of the nodular cases all but eleven (91 per cent) were negative.

Significance of the reaction: The intracutaneous injection of leprosy bacilli produces no reaction in patients who have reached the nodular state, with unlimited proliferation of the bacilli.

The positive reaction appears only in normal individuals resistant to leprosy and in lepers in the neuromacular stage in which a certain degree of resistance is to be presumed. In no case does the negative reaction appear in the normal, tuberculous, or syphilitic.

LYMPHOGRANULOMA INGUINALE, Burney, L. E. Ven. Dis. Inf. **15**: 233, 1934.

Lymphogranuloma inguinale is a specific disease of venereal origin.

Definite proof that the etiologic agent is a filtrable virus has been established.

The chief symptoms and clinical findings are an evanescent primary lesion, not always found, inguinal and iliac lymphadenitis, usually with numerous fistulas running a chronic course of several months to several years, and symptoms of a mild septicemia during the first stage of the lymphadenitis.

In women, because of anatomical difference in lymph drainage, the infection tends to localize in the lymph plexuses around the rectum producing scar tissue and ultimate stricture.

The intradermal test developed by Frei is specific for lymphogranuloma inguinale and from various observations apparently remains positive for many years and probably for life.

Treatment consists mainly of partial extirpation of affected nodes in conjunction with drainage and intravenous use of antimony and potassium tartrate. For those cases presenting rectal structure, surgery is the only recourse.

The disease is not confined to the tropics or even to seaports, but must be looked for in inland cities as well. Of the 4 cases reported, 3 were from Arkansas and 1 from Mississippi.

HODGKIN'S DISEASE, Etiology of: Skin Reaction to Avian and Human Tuberculin Proteins, Steiner, P. E. Arch. Int. Med. **54:** 11, 1934.

Tuberculin protein (Seibert) was used in performing intracutaneous skin tests on thirty-five patients with Hodgkin's disease (lymphogranulomatosis) and on thirty-eight controls with a variety of lymphoma. Tuberculin proteins prepared from both avian and human strains were used in comparative tests for the purpose of obtaining information on the possible etiologic rôle of the avian tubercle bacillus in Hodgkin's disease.

No evidence of specific sensitization to the avian tuberculin protein was obtained in these tests. A marked desensitization (or absence of sensitization) was found to both proteins in the cases of Hodgkin's disease. This relative absence of "normal adult sensitization" was of diagnostic value, especially in the differential diagnosis of tuberculous adenitis. The incidence of absence of sensitization was greater in the group with Hodgkin's disease than in the groups of other lymphomas, which admittedly were small.

The interpretation was made either that (1) the process of Hodgkin's disease desensitizes its victims to these tuberculin proteins or that (2) Hodgkin's disease usually occurs in persons in whom development of the normal sensitization to the tuberculin protein is impossible.

It is difficult to conceive of either of these phenomena as occurring in a disease absolutely unrelated to tuberculosis.

BLOOD, in Healthy Young Infants, Washburn, A. H. Am. J. Dis. Child. **50:** 413, 1935.

The results of 908 total leucocyte counts on twenty healthy infants together with an analysis of 608 differential counts on seven of these infants are presented by means of tables and charts.

The majority (800) of these counts were done between the hours of 9:00 A.M. and 5:00 P.M., the remainder being done during the night. In most instances 8 counts were made in one day, from six to twelve such series being done on each baby during the first three to six months of life.

Concerning the leucocyte counts of the blood of healthy infants from the second to the twenty-sixth week of life the following conclusions seem justified:

The total leucocyte count may vary from 5,000 to 24,000 without indicating the presence of demonstrable disease, but the majority of the counts (the middle 80 per cent) lie between 8,000 and 16,500.

Fluctuations in the counts of all the white blood cells during the hours of the day and night show no tendency to orderly rhythm, vary a great deal from one day to another and show no correlation with age, activity, feedings, or the routine events of the day.

The curve of the lymphocyte counts is the only one which shows a persistent tendency to parallel that of the total leucocyte counts.

The limits of variation which may be expected in each of the main groups of leucocytes are approximately as follows: lymphocytes, from 2,300 to 15,700 per c.mm. (from 27 to 88 per cent); polymorphonuclear neutrophiles, from 500 to 9,500 (from 5 to 63 per cent); all the granulocytes, from 650 to 11,500 (from 10 to 68 per cent); and monocytes, from 150 to 2,400 (from 1 to 18 per cent).

The variation in the differential leucocyte counts of individual infants or in any one infant from time to time is as striking as that previously reported in the total leucocyte counts.

BLOOD, in Healthy Young Infants, Washburn, A. H. Am. J. Dis. Child. 50: 395, 1935.

The routine technic and classification used in differential white blood cell counts have been compared with more specialized methods.

The first study consisted in comparing 100 differential counts on fixed smears prepared with Wright's stain with the same number of counts performed on fresh drop preparations supravitally stained.

Charts are presented which show the variation in the percentage of each cell type in comparing: (1) differential counts on preparations stained with the vital stain and with Wright's stain, (2) a second series of counts on preparations stained with Wright's stain and (3) the average of these two counts on preparations stained with Wright's stain with the counts on preparations prepared with the vital stain.

There was no constant or striking tendency for any one cell type to appear more frequently in the fresh drop preparation than in the fixed smear or vice versa.

The deviations noted in each of the three sets of comparisons were greater than those usually given, being smallest for the comparison of the first and second series of counts on the fixed smears prepared with the Wright stain.

From the study it was concluded that a fixed smear stained with a reliable Wright's stain may show the same distribution of white blood cells in infant's blood as is shown in a fresh drop stained with neutral red and janus green B.

The second study consisted in a critical analysis of the Arneth, Schilling and similar classifications of the granulocytes.

Tables are given to show the variations which were found in a small group of healthy infants when the various methods were used.

The uncertainty of classification as well as the time-consuming nature of the procedure are felt to overbalance any value gained by grouping the neutrophiles according to the number of nuclear lobes.

By Schilling's method the lack of constancy and reliability in classification as well as the amount of variation found are much greater for the stab cell than for the more definitely immature juvenile form or myelocyte.

It was concluded that in the young infant the simplest, safest, and most significant classification of the granulocytes is accomplished by dividing them into groups of myelocytes, immature polymorphonuclears, polymorphonuclears, eosinophiles and basophiles.

For routine work and for most practical purposes in clinical medicine the routine method of staining and counting the white blood cells seems to be as satisfactory as, and safer than, the special methods studied. This is based on the assumption that the smear is carefully made, the Wright stain is of good quality, and the count is done thoroughly and honestly.

SYPHILIS, Congenital, Diagnosis by Means of Dark-Field Examination of Scrapings from Umbilical Vein, Ingraham, N. R. J. A. M. A. 105: 560, 1935.

In this series of cases dark-field examination of the umbilical vein has been instrumental in detecting syphilis in 19 (21.8 per cent) of the 87 living offspring of syphilitic mothers on whom it was tried, with an accuracy which might be considered 100 per cent, and at a time in the infant's life when most other clinical criteria and laboratory aids are unsuccessful. Since 35 of the living infants studied, however, were actually proved by observation to have syphilis, it is needless to say that a negative dark-field examination does not rule out syphilis in the child but merely indicates the necessity of studying the infant by other methods. The fact that in 54 per cent of the diseased children the diagnosis was made within a few hours after birth seems to indicate that this method has a diagnostic value which somewhat overshadows its cumbersomeness. It is truly unpractical to examine a specimen of the cord obtained at every delivery in a large maternity service,

but when the diagnosis of syphilis is made in the mother, as it should be, before delivery takes place, then the suspected syphilitic specimens can be examined without placing too great a burden on the physicians in charge, and the diagnosis of congenital syphilis can be established immediately in a limited number of cases.

TUBERCULIN TESTS, Comparison of, Anzen, G. Am. J. Dis. Child. **50:** 104, 1935.

In the present investigation the plaster and the Pirquet test yielded consistent results in 98 per cent of the cases. In 0.5 per cent of the cases the plaster test gave a positive reaction while the reaction to the Pirquet test was entirely negative. In febrile states the plaster test proved much more sensitive than the Pirquet test. Even when both tests gave positive results the plaster test, in the majority of cases, yielded the more powerful reaction. Were the plaster test alone employed, only 1.25 per cent of the positive reactions would fail to be detected.

BACTERIOLOGY: Simple Method of Supplying Carbon Dioxide in Jars for Bacteriologic Cultures, Thompson, L. Am. J. Clin. Path. **5:** 313, 1935.

The method to be described is very simple and requires no special apparatus. The carbon dioxide is derived from sodium bicarbonate which has been mixed with sulphuric acid. Two solutions are prepared: one, a molar solution of sodium bicarbonate, which is made by dissolving 84 gm. of sodium bicarbonate in water and making the volume up to one liter; the other, a solution of sulphuric acid, which is made by diluting concentrated sulphuric acid so that 1 c.c. of acid is added to 29 c.c. of water. The acid so made is sufficient to react with the bicarbonate solution part for part and still leave a slight excess of acid. Sulphuric acid, being nonvolatile, does not affect the cultures. Each cubic centimeter of the bicarbonate solution should furnish 22.4 c.c. of carbon dioxide.

An ordinary 3 quart museum jar has proved very satisfactory. A rubber ring is used for a seal, but no other precautions have to be taken to prevent loss of carbon dioxide from the jar. The plates that are to be incubated are placed in the jar; then, a sufficient quantity of the bicarbonate solution to produce approximately a 10 per cent concentration of carbon dioxide within the jar is placed in a small wide-mouth bottle. The cover is put in place and an amount of the sulphuric acid solution equal to the amount of bicarbonate solution that is employed is added with a pipette, through a hole in the cover. The stopper is restored to the hole as promptly as possible, and the jar is placed in the incubator.

Erratum

On pages 651 and 652 in the March issue, article by Spohr and Landy, the cuts are transposed over the legends. The cut appearing on page 651 should appear on page 652 over the legend for Fig. 2.